



INDIAN AGRICULTURAL
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ANNALS OF TROPICAL MEDICINE
AND PARASITOLOGY

THE UNIVERSITY OF LIVERPOOL

ANNALS
OF
TROPICAL MEDICINE AND
PARASITOLOGY

ISSUED BY THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Edited by

PROFESSOR WARRINGTON YORKE, M.D. M.R.C.P.

PROFESSOR D. B. BLACKLOCK, M.D.

PROFESSOR W. S. PATTON, M.B.

EMERITUS PROFESSOR R. NEWSTEAD, F.R.S.

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Professor J. W. W. STEPHENS, F.R.S., retired last September, after twenty-seven years' service with the Liverpool School of Tropical Medicine. He was appointed Walter Myers Lecturer in Tropical Medicine in 1903, and from 1913 to 1928 he occupied the Alfred Jones Chair of Tropical Medicine. He has been a member of the editorial staff of this journal from its commencement in 1907.

ANNALS
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TROPICAL MEDICINE AND
PARASITOLOGY

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- (2) Two courses for the Diploma in Tropical Hygiene, commencing on the 12th January and the 26th April. The D.T.H. examinations are held in March and July.
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1923 Fraser, N. D.
1923 Lee, R.
1923 Pierce, E. R.
1923 Raja, Rojaporum
1923 Reid, C. B. B.
1923 Richmond, A. E.
1923 Steven, J. B.
1923 White, Charles Francis
1924 Bilimoria, H. S.
1924 Carson, J. C.
1924 Chopra, B. L.
1924 Davis, B. L.
1924 Hardy, M. J.
1924 Jennings, C. B.
1924 Johnstone, F. J. C.
1924 Keirans, J. J.
1924 Lee, S. W. T.
1924 Macdonald, G.
1924 Maclean, G.
1924 Mathur, W. C.
1924 Mitchell, J. M.
1924 Owen, D. Uvedale
1924 Palmer-Jones, Beryl
1924 Sankeralli, E. J.
1924 Singh, H.
1924 Theron, Elizabeth M.
1925 Adams, Alfred Robert Davies
1925 Ashton, Frank Richard
1925 Ashworth, Esther
1925 Bamford, Charles Walker
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1925 Black, John
1925 Clark, George
1925 Coghlan, Bernard A.
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1925 Ellam, Mary Muriel
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1925 Green, Frederick Norman
1925 Grutu, M. S.
1925 Hawe, Albert J.
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1925 Kerr, James R.
1925 Mackay, Donald M.
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1925 Makkawi, M.
1925 Maldonado, Leopoldo Garcia
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1925 Shah, Khwaja Samad
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1926 Aitken, W. J.
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1926 Austin, T. A.
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1926 Besson, W. W.
1926 Bligh-Peacock, R. N.
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1926 Kamakaka, K. H.
1926 Kennedy, J. H.
1926 Khatri, L. D.
1926 Lennox, D.
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1926 Mackay, A. G.
1926 McLean, N.
1926 MacSweeney, M.
1926 Malhautra, K. L.
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1926 Manuwa, S. L. A.
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1926 Molony, E. F.
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1926 Oppenheimer, F.
1926 Ormiston, W. S.
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1926 Voigt, C.
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ATTEMPTS TO TRANSMIT *LEISHMANIA TROPICA* BY BITE : THE TRANSMISSION OF *L. TROPICA* BY *PHLEBOTOMUS SERGENTI*

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(*Received for publication 17 January, 1929*)

PLATE I

In previous papers (1925 and 1926) it was shown that *Leishmania tropica* is a natural parasite of *Phlebotomus papatasi*, and that the flagellates naturally occurring in this sandfly produce oriental sore when inoculated into man. It was also shown that *L. tropica* ingested from human lesions ex-flagellates in *P. papatasi* and, after undergoing a cycle, produces forms which are infective for man (1926 and 1927). It was further shown (1927) that *P. papatasi* can be infected with different species of *Leishmania* by feeding on cultures through a membrane, and that *L. tropica* from cultures behaves exactly as *L. tropica* ingested from an oriental sore. As *L. tropica* adopts an anterior position in *P. papatasi*, it was concluded that transmission in nature takes place by the bite of the sandfly. It was, therefore, determined to infect sandflies with *L. tropica* by feeding on cultures through a membrane and to attempt transmission by bite on as large a number of volunteers as could be obtained, in order to eliminate possible negative results due to individual immunity. That a complete immunity to *L. tropica*, in the form of L.D. bodies from a human lesion exists, is shown by the following experiment, previously recorded in this journal (1926). Material from an experimental human lesion (a subcutaneous nodule) was inoculated into two human beings (20.4.26). One has remained negative up to date, and the other developed an oriental

sore from which *L. tropica* was cultured (Strain F). The strain obtained from this case was used for infecting sandflies and attempting to infect human beings by bite.

That there is immunity in man to the flagellates of *L. tropica* from artificially infected sandflies is shown by the following facts, also previously recorded in this journal (1927). Out of nineteen volunteers inoculated with *L. tropica* after a development of eight to twenty-one days in the sandfly, six developed oriental sore. The thirteen other volunteers are still negative.

It is also shown (1927) that cultures of *L. tropica* on Locke-Serum-Agar lose their infectivity for man, but that infectivity is restored if the culture is passed through a sandfly. It was, therefore, expected that a positive result would eventually be obtained by feeding the sandflies infected with cultures on human beings.

A strain of *L. Tropica* isolated on 11.8.27, from the above-mentioned case, was employed (Strain F). This strain was used because of its great infectivity for *P. papatasii*. Strains of *L. tropica* vary greatly in their infectivity for *P. papatasii*, and this may explain some puzzling facts in the epidemiology of oriental sore, e.g., in Jericho, where *P. papatasii* is more numerous than in any part of the Middle East we have examined, and, where imported cases are few or none, a few cases of oriental sore occur annually, but the large majority of the population escape the disease. In Bar Elias, a village in Syria, *P. papatasii* and *P. major* are the only sandflies present which bite man, but *P. major* is rare and *P. papatasii* is abundant. During the last six years almost every person in the village has become infected with oriental sore and, accepting the sandfly theory of Leishmaniasis, the only vector to be considered is *P. papatasii*. In Bar Elias we are dealing with a strain which is highly infective for *P. papatasii*, much more so than the strain from Jericho. We hope to discuss this point more fully in a future communication.

A total of 750 laboratory bred sandflies were fed on emulsions varying from 100 to 6,000 per cmm., and of these 708 sandflies became infected. In nine experiments, 124 sandflies were fed on emulsions of from less than 100 up to 500 flagellates per cmm. (i.e., 10 to 50 flagellates per feed), and 91 became infected. In two of these experiments nearly all the sandflies were infected (19 out of 20).

In addition, 69 sandflies fed on emulsions of L.D. bodies, in inactivated normal blood, from selected cultures in medium made up with immune serum, and 26 became infected. There was no difference in the infection rate between emulsions made up with normal and immune serum.

There is evidence that blood is inactivated almost immediately in the sandfly. This evidence rests on the following grounds. Normal blood, both of man and of the rabbit, is strongly lytic for *L. tropica*, *L. donovani* and *L. infantum*. This lytic action depends entirely on complement, and disappears on inactivation. Re-feeding infected sandflies on fresh blood makes no difference to the infection rate and, in the majority of cases, does not influence the intensity of the infection in the sandfly. If sandflies are allowed to feed on an emulsion of *L. tropica* in active blood, where the flagellates are rapidly disintegrating a high infection rate is, nevertheless, produced in the sandflies, proving that the lytic action ceases at once inside the sandfly.

E.g. 18.9.27. An emulsion of *L. tropica* in active rabbit blood was made up to 20,000 cmm. Twelve sandflies fed on this emulsion. After one and a half hours, when all the sandflies had fed, the emulsion was reduced to less than 300 per cmm. Nine of the sandflies were subsequently found positive.

22.9.27. An emulsion of *L. tropica* in active rabbit serum coloured with inactivated blood was made up to 2,000 per cmm. After half an hour the emulsion was too poor to be counted in a haemocytometer (less than 200 per cmm.) ; 45 sandflies fed on this emulsion half an hour after it was made up. Of these, 19 were subsequently found positive, proving that the lytic action of the serum ceased inside the sandfly.

Because of the high infectivity of the strain of *L. tropica* used, the above experiments are not as conclusive as the following one.

18.9.27. An emulsion of a culture of visceral *Leishmania* from a dog in active rabbit blood was made up to 6,000 per cmm. ; 37 sandflies fed on this emulsion. The majority fed within half an hour after the emulsion was made up. The emulsion was found to be reduced to less than 300 per cmm. ; 7 sandflies were subsequently found positive. Had the lysis continued inside the

sandflies as it did in the emulsion, the infection rate would have been almost nil.

Occasionally one finds a rabbit serum which contains heat stable lysins not destroyed by inactivation or by heating to 75° C. for half an hour. Experiments similar to those above showed that the heat stable lysins are also inactive inside the sandflies.

Table I shows the number of positive sandflies (infected from Strain F) fed on each volunteer, the number of days after the infecting feed on culture, and the period during which the feeds took place.

It will be seen from the table that there was a total of 253 feeds by sandflies (*P. papatasii*) subsequently found positive. As most of the sandflies bit from two to five times before completing their feed, there was a total of over 500 bites. Of the 253 feeds, 54 were from six to seven days, 169 from eight to fifteen days, and 30 from sixteen to thirty days after the infecting feed. The sandflies were kept at temperatures of 19° C. to 23° C., and were re-fed at intervals of three days.

In a few cases they were re-fed at intervals of two days. The longest time a positive sandfly, *P. papatasii*, lived was 32 days after the first feed.

EXPERIMENT ON VOLUNTEER NO. 8, WITH *P. sergenti*.

P. sergenti, No. 122. Hatched in laboratory. Fed on case of oriental sore from Artuf, 29.7.28.

4.8.28. Bit volunteer No. 8 on the right forearm, but did not draw blood. There was a local reaction which lasted three days.

Sandflies died two hours after biting. The cardia and the stomach were found heavily infected.

Result: Negative up to date.

The result of the feeding experiments with *P. papatasii* were negative in the case of volunteers 1, 3 to 12, and the puppy (volunteers 1, 2 and 6, were also used for inoculation experiments).

EXPERIMENTS ON VOLUNTEER NO. 1.

Sandfly No. 3606. Hatched in laboratory, 16.11.27. Fed on culture eleven days old, circ. 200 per cmm. Re-fed 21.11.27. Died and dissected 29.11.27. Heavy infection from stomach up to

TABLE 1.

Showing experiments with infected *P. papatasi*.

Days after infection fed	Number of positive sandflies fed on volunteers												Puppy	Total number of sandflies fed on each day
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 12		
6	28	3	31
7	18	3	2	...	23
8	12	...	2	...	8	20	42
9	2	5	3	...	12	22
10	9	1	...	2	4	4	1	21
11	2	3	...	2	...	3	...	5	3	18
12	5	3	2	3	1	3	3	4	24
13	4	3	4	...	2	1	14
14	4	2	...	1	4	1	3	15
15	1	3	2	2	...	3	1	...	1	13
16	2	1	3
17	...	1	...	1	2	...	1	5
18	2	...	1	1	1	5
19	2	1	2	5
20	1	...	3	4
21	1	1	2
22	2	2
24	1	1
26	1	1
28	1	1
30	1	1
Total Number of Sandflies fed on each Volunteer ...	88	15	2	11	19	21	3	67	7	4	7	2	7	253

PERIOD DURING WHICH EXPERIMENTS WERE PERFORMED.

On Volunteer No. 1.	3.9.27 to 8.3.28.	On Volunteer No. 8.	11.12.27 to 26.2.28.
On Volunteer No. 2.	25.9.27 to 8.1.28.	On Volunteer No. 9.	3.1.28 to 13.1.28.
On Volunteer No. 3.	29.11.27.	On Volunteer No. 10.	6.12.27 to 8.12.27.
On Volunteer No. 4.	19.12.27 to 13.2.28.	On Volunteer No. 11.	10.1.28 to 15.1.28.
On Volunteer No. 5.	21.10.27 to 28.10.27.	On Volunteer No. 12.	3.1.28.
On Volunteer No. 6.	31.10.27 to 12.2.28.	On the Puppy.	14.11.27 to 30.11.27.
On Volunteer No. 7.	5.1.28 to 31.1.28.		

middle of the proboscis. Part of the flagellates inoculated into Volunteer No. 1, on left forearm.

Sandfly No. 3607. Details as above. Part of flagellates inoculated into the same scarification as No. 3606.

Result : Negative during observation period of thirteen months.

A sandfly, *P. sergenti* ♀, caught in Baghdad, 27.5.28. Died and dissected 28.5.28. Heavy infection with flagellates found in stomach and cardia.

Flagellates inoculated into two points in the left forearm of Volunteer No. 1.

Result : Negative during an observation period of seven months.

EXPERIMENT ON VOLUNTEER NO. 6.

P. sergenti ♀, No. 106. Laboratory bred. Hatched 27.7.28. Fed on case of oriental sore from Artuf, 27.7.28. Re-fed on guinea-pig, 31.7.28., found dead on morning of 4.8.28. Dissected and found heavily infected. Inoculated on two points on arm of Volunteer No. 6.

Result : Negative up to date.

EXPERIMENTS ON VOLUNTEER NO. 2.

The infected *P. papatasii* were fed on the upper and external part of the left forearm and on an area several inches square, about the middle of the left arm. These feeding experiments were carried out between 25.9.27 and 8.1.28.

On the 11th of November, 1928 (ten months after the last feed by infected sandflies) this volunteer noted two small lesions on the left arm on the site on which infected sandflies had fed. These lesions were examined on 12.11.28, and found to consist of two minute vesicles unlike any oriental sore previously examined. Of the eleven experimental lesions which we have previously recorded, ten commenced as scaly papules, and one as a subcutaneous nodule. As the lesions occurred on a site on which sandflies had fed, they were opened, and smears were made and stained with Giemsa. The vesicles were found to contain a clear fluid full of extra-cellular L.D. bodies. On the 13th two more vesicles appeared in the neighbourhood of the first ones. These were examined on the 14th, and one was found positive.

107 sandflies, *P. papatasii*, laboratory bred, fed on this case and

all were subsequently found negative. Only about a third of the sandflies fed directly on the lesion, and the remainder fed in the neighbourhood of the lesions.

It seemed certain that these lesions were caused by flagellates of *L. tropica* introduced into the skin by the bites of one or more of the infected sandflies, but a slight element of doubt appeared. It was found that during the summer of 1928 Volunteer No. 1 lived in the Bokharian quarter of Jerusalem, a quarter where there are always a number of imported cases of oriental sore from Baghdad, Aleppo and Persia and where, moreover, sandflies are very common. Although the lesions appeared on the site on which artificially infected sandflies had fed, nevertheless the possibility of the lesions being natural ones must be considered, particularly as several locally acquired cases of oriental sore have been noted in Jerusalem, in 1928. The experiment cannot, therefore, be regarded as an absolutely conclusive proof of the transmission of oriental sore by the bite of *P. papatasi*.

OTHER INOCULATION EXPERIMENTS ON THE SAME VOLUNTEER.

Experiment No. 1. 23.7.28. Culture material of *L. tropica* was inoculated into the tails of four mice, by the method recommended by Parrot and Donatien (1927), who demonstrated that mice are very sensitive to this method of inoculation with *L. tropica*. The same strain was inoculated into two points on the lower part of the left arm of Volunteer No. 2.

Result : Volunteer No. 2 has remained negative on the site of the inoculated points within an observation period of five months. Two mice were found positive on the inoculated sites after ten days. (Further experiment showed that mice inoculated by this method show L.D. bodies on the inoculated sites as early as five days after inoculation.)

Experiment No. 2. 2.8.28. *P. sergenti* ♀, hatched in laboratory on 27.7.28. Fed on the same day on case of oriental sore from Artuf. Sandfly kept at 27° C. Died and dissected 2.8.28.

Flagellates from the mid-gut were inoculated into three scarified points on the right arm of Volunteer No. 2.

Result : On 23.11.28 a raised spot was noted on the site of one of the scarifications. On examination, L.D. bodies were found.

Experiment No. 3. *P. sergenti*, No. 232. Hatched in laboratory, 7.8.28. Fed, 8.8.28, on case of oriental sore. Re-fed 12.8.28.

16.8.28. Refused to feed. Died and dissected. Was found to be heavily infected. Flagellates inoculated into two points on the left deltoid region.

Result : Negative up to date.

The lesion on the right arm appearing actually on the scar of a previous inoculation with flagellates from an artificially infected *P. sergenti*, proves that after six days at 27° C. *L. tropica* in *P. sergenti* is infective for man. In this case the possibility of natural infection is negligible for, whereas sandflies might feed on a site several inches square, it is improbable that a wild infected sandfly should feed on a single experimental point. In the case of *P. papatasii* it was shown that *L. tropica* becomes infective for man after eight days at 19 to 23° C.

THE BEHAVIOUR OF *L. TROPICA* IN *P. SERGENTI*

Sinton (1925) first suggested *P. sergenti* as a possible vector of oriental sore, after studying the distribution of the sandfly and the disease. Evidence obtained by a study of distribution of sandflies must be interpreted very cautiously. Oriental sore is absent in places where both *P. papatasii* and *P. sergenti* occur. We will deal with the distribution of sandflies and oriental sore in another communication. For the present we will confine ourselves to experimental facts obtained by feeding *P. sergenti* on cultures of *L. tropica* and on oriental sore.

P. sergenti does not feed readily through membranes and under laboratory conditions we were not very successful in feeding this sandfly on man. Only a small proportion, varying from none to 25 per cent. of laboratory bred *P. sergenti* were induced to feed on man and, working in Baghdad, we never succeeded in feeding wild *P. sergenti* on man although, in nature, these sandflies feed mainly on human beings. It was noted that *P. sergenti* feeds more readily on injured than on normal skin, while *P. papatasii* shows no selection and feeds equally well on both normal and injured skin. For the purpose of breeding *P. sergenti* we were compelled to leave sandflies all day in a cage containing a guinea-pig, and even then not more than about 10 per cent. of the sandflies fed. Fortunately, *P. sergenti*

lays eggs after one feed. Out of over 200 *P. sergenti* offered a feed on normal human skin, only five fed, whereas 25 out of 200 fed on oriental sores.

Owing to the kindness of Dr. A. E. Mills we were able to carry out the following experiments in the Central Laboratory, Baghdad.

TABLE II.
Experiments with membranes.

Sandfly	Date of feed	Number of flagellates per feed	Died	Species	Result
No. 1	19.5.28	20 circ.	22.5.28	<i>P. sergenti</i>	Heavy infection in stomach.
No. 2	19.5.28	20 circ.	22.5.28	<i>P. sergenti</i>	Slight infection in cardia.
No. 3	19.5.28	20 circ.	20.5.28	<i>P. papatasi</i>	Negative.
Nos. 4-10	19.5.28	20 circ.	22.5.28	<i>P. papatasi</i>	Heavy infections in stomach.
No. 11	20.5.28	20 circ.	22.5.28	<i>P. sergenti</i>	Negative.
No. 12	20.5.28	20 circ.	24.5.28	<i>P. sergenti</i>	Heavy infection in stomach. Slight infection in cardia.
No. 13	20.5.28	20 circ.	24.5.28	<i>P. sergenti</i>	Slight infection only in cardia.
No. 14	20.5.28	20 circ.	24.5.28	<i>P. sergenti</i>	Negative.

The sandflies were all caught in Baghdad. They were kept at 30° C.

The experiments, though few, are conclusive. They show that *L. tropica* once established in *P. sergenti* tends to the anterior position even if the infection produced in the sandfly is very slight. *P. sergenti* is, therefore, a probable carrier of oriental sore.

THE INFECTION OF *P. SERGENTI* ON ORIENTAL SORE

The sandflies used in the following experiments were laboratory bred from eggs laid by wild sandflies caught in Baghdad. They were infected in Mosul on a case of locally acquired oriental sore, and were subsequently transported to Baghdad, where they were examined. During six of the eighteen hours' trip to Baghdad, they were subjected to a temperature of about 40° C. in an open car. The sandflies were kept in tubes encased in moistened lint. As will be seen from the following table, the high temperature during six hours had no deleterious effect on the development of *L. tropica*.

TABLE III.

Feeding Experiments with *P. sergenti* on oriental sore in Mosul.

Sandfly	Hatched	Fed	Died	Results
No. 1	2.7.28	4.7.28	7.7.28	Cardia and stomach heavily infected, attachment of medium and long forms to cardiac valve.
No. 2	2.7.28	4.7.28	7.7.28	Cardia and stomach heavily infected, attachment of medium and long forms to cardiac valve.
No. 3	3.7.28	5.7.28	7.7.28	Heavy infection in stomach, long forms.
No. 4	3.7.28	5.7.28	7.7.28	Slight infection in stomach.
No. 5	3.7.28	5.7.28	7.7.28	Heavy infection in stomach.
No. 6	3.7.28	5.7.28	7.7.28	Many ex-flagellating forms in stomach, mostly medium and short forms.
No. 7	3.7.28	5.7.28	7.7.28	Many ex-flagellating forms in stomach, mostly medium and short forms.
No. 8	3.7.28	5.7.28	7.7.28	Negative.
No. 9	5.7.28	6.7.28	7.7.28	Ex-flagellating forms in stomach.
No. 10	5.7.28	6.7.28	7.7.28	Negative.
No. 11	5.7.28	6.7.28	7.7.28	Negative.
No. 12	5.7.28	6.7.28	7.7.28	L.D. bodies and ex-flagellating forms in stomach.
No. 13	3.7.28	4.7.28	8.7.28	Negative contaminated.
No. 14	3.7.28	4.7.28	8.7.28	Heavy infection in cardia, stomach and hind-gut. Long forms.
No. 15	3.7.28	5.7.28	8.7.28	Heavy infection in stomach and cardia. Long forms.
No. 16	3.7.28	6.7.28	9.7.28	Negative.
No. 17	3.7.28	6.7.28	9.7.28	Negative.

It will be seen from the above table that *L. tropica* ex-flagellates and multiplies rapidly in *P. sergenti*. It ascends the cardia within three days. Of seventeen *P. sergenti*, eleven became infected and, of two *P. papatasii* fed on the same sore, one was found infected.

Laboratory bred *P. papatasii* and *P. sergenti* were fed on two cases of oriental sore from Dr. A. Dostrowsky's Clinic at the Rothschild Hospital, Jerusalem.

Case I was acquired in Baghdad and was, therefore, transmitted by *P. sergenti* or by *P. papatasii*.

Case II was acquired at Artuf, in Palestine, where *P. papatasi* is very common, and *P. sergenti* has never been found. Between every series of feeds, Case II had an injection of Stibosan. Smears were taken at regular intervals, and it was found that the parasites progressively diminished. It will be seen from Table IV that between 27.7.28 (when L.D. bodies in the lesion were very numerous) and 8.8.28, the infection rate in *P. papatasi* diminished markedly, while that of *P. sergenti* was hardly affected. It is, therefore, obvious that *P. sergenti* is a more suitable host for the development of the strain of *L. tropica* from Case II than even *P. papatasi*, though the organism behaves similarly in both sandflies. In three out of eight specimens of *P. sergenti*, which died six days after the infecting feed, flagellates were found in the proximal two-thirds of the proboscis. Out of sixteen specimens of *P. papatasi* which died six days and more after the infecting feed, the proboscis was found infected only in two cases. In one case, in addition to the anterior infection, flagellates were found attached in the hind-gut of *P. sergenti* and the rectum was also heavily infected.

TABLE IV.

Experiments with *P. papatasi* and *P. sergenti* on oriental sores.

Case	Date of feed	Number of <i>P. sergenti</i> fed	Number positive	Number of <i>P. papatasi</i> fed	Number positive
I.	22.7.28	1	1	30	1
I.	25.7.28	0	0	35	1
I.	27.7.28	0	0	2	0
II.	27.7.28	9	5	23	10
II.	29.7.28	3	3	16	4
II.	1.8.28	0	0	36	5
II.	5.8.28	3	1	35	3
II.	8.8.28	6	4	41	3
II.	12.8.28	3	1	33	6
II.	15.8.28	0	0	34	4
Total	25	15	285	37

The sandflies were kept at a temperature of 27° C.

These observations cannot be generalised for all strains of *L. tropica*, because strains vary enormously in their infectivity for *P. papatasi*. It is probable that they also vary in their infectivity for *P. sergenti*. As an example of the variation in the infectivity of strains of *L. tropica* for *P. papatasi*, the following records will be sufficient.

Strain from Tunis. This strain was presented by Professor Nicolle, Director of the Pasteur Institute, Tunis.

TABLE V.
Behaviour of the strain of *L. tropica* from Tunis in *P. papatasi*.

Date of experiment	Age of culture	Flagellates per 0.1 cmm.	Number of sandflies fed	Number positive	Remarks
13.1.28	Days 14	80	22	19	Dissected 4 to 8 days after infecting feed.
15.1.28	5	1,000	5	5	Dissected after 4 to 5 days.
18.1.28	8	1,000	10	8	Dissected after 2 to 6 days.
23.8.28	7	600	30	28	Dissected after 4 to 8 days.

Strain of *L. tropica* isolated from oriental sore in Baghdad.

TABLE VI.
Behaviour of the strain of *L. tropica* from Baghdad in *P. papatasi*.

Date of experiment	Age of culture	Flagellates per 0.1 cmm.	Number of sandflies fed	Number positive	Remarks
27.7.28	Days 8	50	14	0	Dissected 4 to 5 days after infecting feed.
29.7.28	10	80	25	2	Dissected after 4 to 6 days very slight infections.
5.8.28	7	1,000	9	4	Dissected after 4 to 9 days.
7.8.28	6	250	18	0	—
9.8.28	8	250	20	0	—

The strain of *L. tropica* isolated from a lesion on the left arm of Volunteer No. 2 was also only very slightly infective for sandflies. This strain showed an additional peculiarity in that it tended to die out in the sandfly, e.g.:—3.12.28. 27 sandflies fed on an emulsion of 1,600 flagellates per 0.1 cmm. from a culture nineteen days old. Of 8 sandflies dissected within three days after the infecting feed, 6 were found positive. Of 19 sandflies dissected from four to ten days after the infecting feed, only 2 were found positive.

It is necessary to inquire why the results of feeding experiments with so many heavily infected sandflies gave negative results. We think that the reason is that at a temperature of 19 to 23° C., only a small proportion of sandflies acquire an infection in the proboscis. The pharynx is often completely choked and from here flagellates enter the buccal cavity. The posterior part of the buccal cavity may also be plugged with flagellates but, unlike *P. argentipes* infected with *L. donovani*, the whole buccal cavity does not, as far as our observations go, become completely choked. (We have seen complete blocking of the buccal cavity of *P. papatasii* only in one instance. The sandfly was infected from culture of a strain of *L. infantum*.) Flagellates dribble down from the buccal cavity into the proboscis and they may pass almost up to the tip of the proboscis. Sections of some heavily infected sandflies showed flagellates in the coelom and muscle spaces throughout the body and appendages, but not inside the ova. This condition appears to be without significance with regard to transmission. It is not known for how long an insect is viable in this state; the condition is possibly produced shortly before death or during the process of dying. We found later that at higher temperatures (27 to 30° C.) proboscis infections in *P. papatasii* are much commoner but, in the experiments recorded above, the sandflies were kept at 19 to 23° C. This is the probable reason for so many failures in our attempts to transmit by bite. Apart from temperature there may be other factors in wild infected sandflies which have not been reproduced in the experimental ones.

The following method was used to determine whether flagellates can leave this sandfly via the proboscis. Sandflies were infected on cultures and were re-fed at various intervals. After eight days they were allowed to feed through a membrane at room temperature,

on inactivated defibrinated blood which was subsequently examined in fresh preparations and by culturing on Shortt's N.N.N. In six experiments in which a total of seventeen sandflies with eight to seventeen days' old infections fed through membranes, the result was negative. In two experiments in which infected sandflies were kept at 37° C. for half an hour before being placed in the feeding apparatus, positive results were obtained.

Experiment I. Two sandflies fed 22.II.27 on emulsion of *L. tropica* (4,000 flagellates per cmm.), re-fed on puppy 27.II.27. Re-fed on a solution of haemoglobin through membrane, 30.II.27.

After the feed a part of the fluid from the membrane was sown on two tubes of Shortt's N.N.N., and the remainder examined in fresh preparations.

Result: In fresh preparations three solitary flagellates were found. The tubes of N.N.N. were subsequently found contaminated.

The two sandflies died 3.I2.28. Both were found heavily infected. In one the upper part of the cardia was choked and no flagellates were found in the stomach. In the other the cardia was choked and the stomach was also heavily infected. In neither sandfly was the proboscis found infected although the proboscis of one or both sandflies was probably infected on 30.II.27.

Experiment 2. Seven sandflies fed 19.I.28 on an emulsion of *L. tropica*, 3,000 flagellates per cmm. Sandflies kept at 37° C. 23.I.28 re-fed. 27.I.28. Three sandflies re-fed through a membrane on inactivated rabbit blood. A part of the fluid from the membrane was sown on a tube of Shortt's N.N.N.-Agar. The remainder was examined in fresh preparations. In nine preparations not a single flagellate was found.

Result: 2.2.28, the tube of Shortt's N.N.N. was examined and found positive. The culture was continued and found infective for sandflies no less than the parent strain (sixty sandflies fed and fifty-seven became infected). The strain was passed through a mouse and cultured and was still found to maintain its infectivity (147 sandflies fed and 129 became infected).

One of the sandflies died 30.I.28, and the stomach and cardia were found heavily infected. The second sandfly died on 31.I.28, and was fixed in Carnoy. Sections showed a heavy infection in pharynx, cardia and stomach. The third sandfly re-fed on 31.I.28,

was killed immediately after the feed, and was found heavily infected in cardia and stomach.

The positive result obtained was quite unexpected as it was thought that flagellates in the proboscis are incapable of dividing until they enter a vertebrate host.

In neither of the positive experiments could flagellates from the rectum have entered through the membrane, because in the feeding apparatus the rectum points obliquely downwards during the act of feeding. Even if flagellates would have been ejected on the membrane, it is impossible to believe that they could have penetrated into the fluid on the other side. The flagellates could, therefore, have entered the fluid above the membrane only through the proboscis.

In both positive experiments only very few flagellates passed into the fluid during the act of feeding, in spite of the enormous infection in the sandflies. It was thought that flagellates might pass to the end of the proboscis and enter the duct of the hypopharynx from where they would be bound to enter the wound during the act of biting, but neither in dissected or sectioned specimens were flagellates found in the duct of the hypopharynx.

We think there is a greater likelihood of obtaining a positive result by the bite of a sandfly if the latter is kept at a temperature higher than 22° C. Experiments are now in progress with sandflies kept at 27° and 30° C.

In seeking for a possible insect vector of the Leishmanias of man, we must bear in mind that these organisms do not live long together with bacteria. Thus, insects with a rich intestinal flora, such as the housefly, can be safely excluded as possible vectors. Mosquitos often contain bacteria in their alimentary tract. Bed bugs can be excluded as carriers because they do not occur in Baghdad. Unfortunately, the intestinal flora of biting insects has not been sufficiently studied. The alimentary tract of *P. papatasi* and *P. sergenti* is bacteriologically sterile. Although the larvae constantly devour bacteria, yet the adult never contains bacteria in its alimentary tract. Sterilisation apparently occurs during pupation. Bacteria which accidentally invade the alimentary tract of the sandfly and multiply usually cause the death of the insect in a few days. *P. papatasi* and *P. sergenti* are, therefore, favourable hosts for *L. tropica*.

SUMMARY AND CONCLUSIONS

Two hundred and fifty-three sandflies, *P. papatasi*, heavily infected with *L. tropica* from cultures fed on twelve human beings and a puppy.

There were more than five hundred bites, but the puppy and eleven volunteers remained negative during an observation period of one to fourteen months.

One case developed *L. tropica* on one of the areas on which infected *P. papatasi* fed, but natural infection is not excluded as he lived in a quarter where oriental sore occurred.

It has been shown by membrane experiments that *L. tropica* may leave an infected *P. papatasi* via the proboscis.

L. tropica ex-flagellates in *P. sergenti* and undergoes a cycle of development similar to that in *P. papatasi* and, after six days at 27° C., forms appear which are infective for man.

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PLATE I

EXPLANATION OF PLATE I

Sagittal section through the clypeus and mouth-parts of *Phlebotomus papatasi*, showing mass of flagellates of *L. tropica* between epipharynx and mandible (in the food canal).

Strain *F*. Infection fourteen days' old. Magnification 315 \times .

B.C. = Buccal cavity.

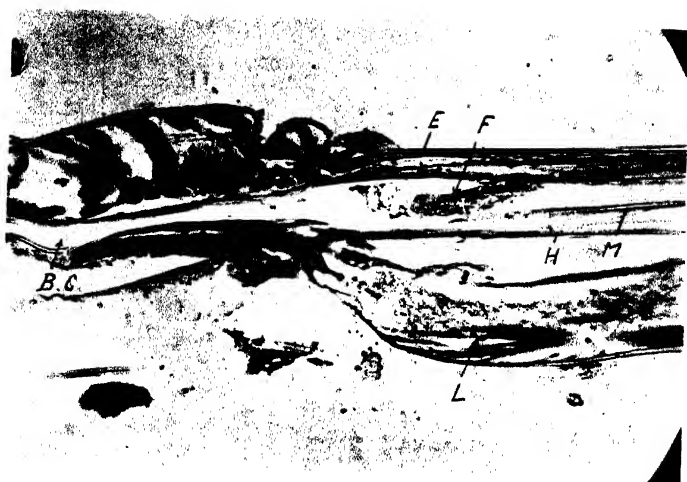
E. = Epipharynx.

M. = Mandible.

H. = Hypopharynx.

L. = Labium.

F. = Flagellates of *L. tropica*.



ADDITIONAL EVIDENCE ON THE OCCURRENCE OF *L. TROPICA* IN WILD *PHLEBOTOMUS PAPATASII*

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The authors (1925 and 1926) recorded three experiments in which *Herpetomonas* from naturally infected sandflies, *Phlebotomus papatasii*, caught in Jericho, were inoculated into man and produced oriental sores in two cases and a subcutaneous nodule containing L.D. bodies in one case. The diagnosis of *P. papatasii* was made because no males of other species which bite man were found in Jericho and at the time the work was done no characters of specific value for females of the genus *Phlebotomus* were known.

Subsequently (1926) the authors described the diagnostic value of the pharynx in *P. papatasii*.

Sinton (1927) criticised the diagnosis of *P. papatasii* made by the authors, and pointed out that a diagnosis based on negative and not on positive evidence was not conclusive.

Knowles (1928) stated that, working in Calcutta, Smith failed to infect laboratory bred *P. papatasii* by feeding them on oriental sores rich in L.D. bodies. Knowles implied that the positive results recorded by other authors were probably obtained with *P. sergenti* wrongly diagnosed as *P. papatasii*.

This criticism is not applicable to the experiments recorded by the authors (1927), in which *L. tropica* was transmitted to man from artificially infected sandflies, for in these experiments laboratory bred sandflies were used and the pharynx was regularly examined. (The diagnostic value of the pharynx was discovered towards the end of 1925.)

Strains of *L. tropica* vary greatly in their infectivity for *P. papatasi*. While some strains produce a high infection rate even when a small number of parasites are ingested, others produce a slight infection rate even when very large numbers of parasites are ingested. Smith's experiments prove that some strains of *L. tropica* are completely non-infective for *P. papatasi*.

Although Larousse (1924) has recorded *P. sergenti* from Palestine, we have never come across a single specimen of this species during four and a half years' collecting. In 1926, 1927 and 1928 we bred about 4,000 sandflies from females caught in Jericho, over 8,000 from females caught in Jerusalem, and almost 1,000 from females caught in Rosh Pinah (near Lake Tiberias), and we have never once seen a single specimen of *P. sergenti* in this material. Nevertheless, Sinton's criticism is legitimate as far as the experiments with *Herpetomonas* from wild sandflies caught in Jericho are concerned, for there is a possibility, however slight, that *P. sergenti*, when present in small numbers, may be overlooked. This possibility is indeed very slight, for the male of *P. sergenti* is so characteristic that it can be diagnosed readily with the naked eye even when it is sitting on a wall. The female, however, can only be identified by the character of the pharynx and spermathecae.

In the case of the wild sandflies used for inoculation experiments, a part of the material was fixed on slides, stained and mounted. The slides were recently re-examined and the head of only one sandfly was found. This sandfly was caught in Jericho, 8.9.25, and was dissected 9.9.25. Flagellates were found in the oesophagus, oesophageal diverticulum, mid-gut and hind-gut. Part of the flagellates was inoculated into a human being and produced a subcutaneous nodule containing numerous L.D. bodies. Cultures were obtained from the lesion and the strain has been maintained continuously on Locke-Serum-Agar. Although the lesion was atypical, there is no doubt that it was caused by *L. tropica* for direct inoculation from this lesion into a human being produced a typical oriental sore.

Examination of the mounted head showed a *pharynx* typical of *P. papatasi*. The occurrence of *Leishmania tropica* infective for man in wild *P. papatasi* is, therefore, proved conclusively.

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**TERNIDENS DEMINUTUS (RAILLIET & HENRY)
AS A PARASITE OF MAN IN SOUTHERN
RHODESIA ; TOGETHER WITH OBSERVATIONS
AND EXPERIMENTAL INFECTION STUDIES
ON AN UNIDENTIFIED NEMATODE PARASITE
OF MAN FROM THIS REGION**

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(Received for publication 24 January, 1929)

PLATE II

In October, 1927, Dr. X., an American Medical Missionary, was referred to the writer for an opinion on the intensity of a 'hookworm' infection which the patient harboured and which, it was considered, might have been responsible for his 'run down' condition.*

The patient had spent more than twenty-five years in medical missionary work in Portuguese East Africa (Mozambique) and in the eastern portion of Southern Rhodesia. Within the past few years he had commenced the examination of the stools of many of his patients for parasites and had been able to show that 'hookworm' infection, previously unrecognised in this locality, was widespread (ca. 82 per cent.). The attention of the Government public health laboratory was drawn to this high incidence of infection, and some worms secured by Dr. X. after anthelmintic treatment of a native with carbon tetrachloride were submitted to the laboratory. The situation has been reported upon briefly by the medical director under the paragraph heading 'Hookworm' in the 'Report of the Public Health for the Year 1925, Southern Rhodesia' (p. 19).

In September, 1926, our patient had made an examination of his own stools, and had found a few nematode eggs which he con-

* The writer is indebted to Dr. George C. Shattuck, for the opportunity of examining the patient, and also to the patient for his hearty co-operation in prosecuting these studies.

sidered similar to those found in the faeces of patients at the mission station. These eggs he believed to be those of *Necator americanus*.

In our laboratory, *fresh* stools were examined by the Willis-Molloy salt flotation method which furnished a small number (3 to 10 per preparation) of Strongyle eggs. The eggs (see Plate II, fig. A and photomicrograph, Text-fig. 1) are thin-shelled, and somewhat asymmetrical, being slightly less convex on one side than on the other. One pole is usually seen to be more pointed than the other. Immediately after passage from the body, the eggs are in an advanced state of segmentation, 16 or more cells having already been formed. The eggs range in size from 72μ to 103μ by 37μ to 45μ . The mean measurement of 30 eggs taken over a period of months was 84.5μ by 41.3μ .



FIG. 1. Photomicrograph of egg found in fresh faeces. $\times 450$.

In consideration of the fact that the eggs of the parasite under investigation were (1) considerably larger than those of either *Ancylostoma duodenale* or *Necator americanus*, (2) that they were markedly different in shape, and (3) that they were deposited in a more advanced stage of cleavage, the writer was convinced that the infection was distinct from the usual hookworm infections of man.

Our first opinion was that the infection might be with a species of *Trichostrongylus*, such as *T. instabilis*, *T. vitrinus*, etc., which have on several occasions been encountered in man in Africa. The possibility of an *Oesophagostome* infection was also to be considered. To establish the identity of the parasite and to procure information of its life-history, a systematic study of the infection was undertaken. Infection experiments were planned, since our patient, who was on furlough in the United States, expected to be ordered abroad in a short time. In the meanwhile a letter had been written to the mission station in Rhodesia requesting that samples of preserved stools of infected natives be forwarded, together with

the few worms that had previously been retrieved from a patient there after treatment. The following notes summarise our observations on the biology and life-history of the parasite.

Development of the egg.—Entire stools were cultured in the ordinary way after mixing thoroughly with a generous quantity of animal charcoal. At room temperature the development of the larva within the egg is completed in about thirty hours. On emerging from the egg, the larva is of the typical rhabditiform type (Plate II, fig. B). It measures about 320μ in length and is 18μ broad near the middle of the body. The buccal cavity is cylindrical and about 12.5μ long, this being approximately equal to the breadth of the body at the level of the base of the buccal cavity. The oesophagus is 0.14 mm. long. The distance between the anus and tip of the tail is 43μ , and the tail tapers in a uniform manner to an acute point. There appear to be no outstanding morphological differences between this larva and the corresponding larvae of the common human hookworm.

After a short period of feeding in the open, which is accompanied by a certain amount of growth, the larva undergoes an ecdysis. The early stage larva is indistinguishable from the first stage. Growth proceeds and the passage from the second to the third, or infective, stage is marked by a metamorphosis which chiefly affects the structure of the buccal region and oesophagus of the larva, transforming the latter from the rhabditiform type to the simple claviform or filariform type. *The infective or filariform larvae* develop in cultures incubated at 30°C . in from 72 to 96 hours. They range in size from 700μ to 780μ in length by 20μ to 21μ in greatest breadth. The morphology of this larva is illustrated in Plate II, figs. D 1 and D 2. It may be distinguished from the infective larva of the hookworms in that it is at least from one-sixth to one-third longer and its oesophagus is relatively smaller, being in this case little more than one-tenth the total body length. Morphologically, very distinct differences can be observed in the finer structure of the anterior end, and in the structure of the slight swelling at the posterior extremity of the oesophagus. A very characteristic peculiarity, which readily serves to distinguish this larva from the corresponding larvae of the hookworms and related human parasites, is the shape of the caudal extremity. In the hookworms the caudal

region tapers gradually and uniformly to an acute point. In the present form, the tail region tapers gradually, but terminates abruptly in a truncated stump (Plate II, fig. D2).

Biology of the Infective larva :—The third stage larva, like the corresponding hookworm larva, remains ensheathed in the cuticle of the second stage larva. But, unlike the hookworm larva, this sheath is retained, apparently, throughout the period of free life and it furnishes the larva with a high degree of resistance against desiccation. When water containing larvae has almost completely evaporated (as in a watch-glass that is exposed) the larvae commence to migrate up the side of the vessel in an endeavour to escape to a moister ambient. On finding themselves in an environment free from moisture, the larvae curl up in a characteristic watch-spring coil. They may frequently be found in masses on the sides of a watch-glass from which the water has evaporated. In an atmosphere which is relatively humid, such coiled larvae remain alive for a considerable length of time. Thus an old faecal culture exposed to desiccation for more than seven weeks so as to become quite dry yielded many larvae when placed in the Baermann isolating funnel. Larvae which had been permitted to become completely desiccated and to remain exposed on a slide for three days were, when re-examined, seen to be in a much shrivelled condition with the internal organs vacuolated. The larvae straightened out on the addition of water, and in about an hour were moving in a perfectly normal manner. This may be contrasted with the great sensitiveness that the larvae of the hookworms exhibit to desiccation even for a matter of a few minutes. The movement of the larva is considerably more sluggish than the active swimming of the infective larvae of the hookworms. They are practically unstimulated by the application of moderate warmth and consequently their isolation from cultures by the Baermann method is unsatisfactory. This method of isolation was, however, used since it was more practical with the lightly infected cultures that we were using than the alternative method of trapping larvae that migrate from cultures into surrounding water. Cultures placed in the isolating funnel were still yielding larvae after two days.

The sluggish movement of the larva, together with its lack of marked thermotropism suggests clearly that infection of the host

is by way of the mouth rather than by the penetration of the skin, as normally occurs with the hookworms. Unfortunately, larvae were secured in such small numbers that experiments to test their ability to penetrate the skin were not warranted. An attempt at the experimental infection of a rabbit was without result. Attempts were made at the experimental infection of dogs and of a monkey, but mature parasites were not found at our subsequent examination of the alimentary tract of these animals.

A HUMAN INFECTION EXPERIMENT

Since the departure of the patient representing the source of our material was imminent, and in order to avoid the loss of further opportunity to study this unusual parasite, the voluntary infection (to a light degree) of a human subject was decided upon prior to attempting to dislodge the parasites in the original infection by anthelmintic treatment for identification. On January 7, 1928, twenty-seven young filariform larvae were placed in a hard gelatine capsule and swallowed. Frequent examination of the stools by concentration methods had been negative, when, on January 30th, an additional fifty active larvae were swallowed in water. Six examinations were made later, but eggs were found in the stools for the first time on March 21st. These eggs were identical with those from the original infection. The infection has persisted now for a period of about nine months, without any apparent decrease. The number of ova in the stools is very small, indicating that the infection is a very light one. No untoward signs or symptoms are evident.

At this point in our investigations, in response to our request, we received a small sample of formalin preserved faeces of an infected native from Southern Rhodesia, together with eight worms which had been saved from stools washed after treatment. On examining the faeces, several eggs were found similar in size and shape to those which we had been studying. All of the eight worms (of which two were males) on examination proved to be *Ternidens deminutus* (Railliet and Henry, 1905). In consideration of this we felt justified in expressing some doubt as to the general 'hookworm' infection in this region being due to either *Ancylostoma* or *Necator*.

ATTEMPTS TO SECURE THE PARASITE FOR IDENTIFICATION BY TREATMENT

Seeing that some degree of success had been secured in Rhodesia in expelling the parasite with carbon tetrachloride, we decided to use the same drug in the present case. Since this drug, as a rule, has a very low toxicity, 4 c.c. instead of the usual 3 c.c. was administered on an empty stomach after preliminary purgation with magnesium sulphate. The drug was well-tolerated. Stools, immediately after passage on the first day, were very carefully washed through a fine screen without any worms being recovered. They were likewise washed on the three days following with the same disappointing result. A week later and on several subsequent occasions eggs were found in the stools in apparently undiminished numbers. The patient then left the country.

Some time later, an attempt to retrieve the parasites from our experimentally infected case was made. After preliminary purging, 5 c.c. of tetrachlorethylene were taken in a hard gelatine capsule. The stools were washed for three days through a fine sieve, but no worms were recovered. The eggs still appear regularly in the faeces.

On the basis of evidence already available, it appears probable that *Ternidens deminutus*, like species of *Oesophagostomum*, *Triodontophorus*, etc., is a form which, at least in its early life, inhabits cysts in the mucosa and wall of the large intestine (caecum and colon). If this is later substantiated, since it is not unusual to find that anthelmintics are effective only against parasites which live exposed in the lumen of the small intestine, the negative results following the treatment in our cases may be accounted for.

THE PROBABLE IDENTITY OF THE PARASITE

The failure of our anthelmintic to dislodge the parasites in the infection has prevented the possibility of making a specific determination. Until an opportunity is obtained to follow an infection to necropsy and in this way to secure the adults, or to obtain a richer source of material for infection experiments in apes, we can do no more than suggest the probable identity of the parasites.

Although the eggs are definitely those of a Strongyle nematode,

there is ample evidence to prove that it is neither *Ancylostoma* or *Necator*. The size of the eggs is such that Trichostrongylids cannot be eliminated from consideration, but there appears to be no further evidence to incriminate a species of this group of parasites. The possibility of the infection being due to a species of *Oesophagostomum*, such as have on occasion been found to parasitise man, may be ruled out, since the eggs of these species are slightly smaller and are not asymmetric in shape. Further, the tail in the rhabditiform larva and in the infective larvae of *Oe. apiostomum* (= *Oe. brumpti* in the opinion of Leiper) is figured and described by Walker (1913) as being very elongate and filiform. This characteristically attenuated tail is also observed in the larva of *Oe. dentatum* of the pig, according to Goodey (1924) and *Oe. columbianum* of sheep, according to Veglia (1924). This is in contrast with the larvae secured from the present infection.

The material of *Ternidens deminutus* received from Rhodesia is, unfortunately, such that even when a female specimen was teased apart, eggs were not obtained, so that the direct comparison of the eggs cannot be made. However, Leiper (1908) gave the measurements, 60μ to 80μ by 40μ , as the size of the eggs in the uterus of preserved female *Ternidens deminutus*. The larger range in Leiper's measurements agrees very well with the mean size of the eggs in the infection we have studied. The shape of the eggs, although not well depicted by Leiper, also seems to be in agreement. Evidence to supplement the idea that the infection which we have been investigating is *Ternidens deminutus* is to be found when we review the previous records of infection with this parasite and its geographic distribution in man.

Railliet and Henry (1905), while examining the collection of parasitic nematodes in the Museum of Natural History in Paris, found a vial containing parasites collected in 1865 at autopsy of a native of Mayotte, in the Comoro Islands, off the coast of Portuguese East Africa. The patient had succumbed to an anaemia, and in the intestine, the walls of which were 'considerably thickened and presented numerous petechial hæmorrhages of the mucosa, nematodes were found. The medical officer who had performed the autopsy identified these as *Ancylostoma duodenale* and considered them the cause of the clinical condition. In this vial, Railliet and Henry found two nematodes, a male and female, which were new to science

and for which the name *Triodontophorus deminutus* was proposed. Later the same authors, on revising the classification of the Strongylidae, withdrew the parasite from the genus *Triodontophorus* and erected the new genus *Ternidens* for its reception, placing the genus in the sub-family Oesophagostominae. Up to the present this genus is represented by a single species.

The second record of *T. deminutus* is by Leiper (1908), to whom Turner, a medical officer on the mines of the Witwatersrand (Transvaal) sent some hookworms recovered at autopsy from the intestines of two natives of Nyassaland. Turner noticed that among the worms there were a few females, slightly larger than the others, occupying what he thought to be an abnormal habitat, the large intestine, the mucosa of which showed congested patches. These worms were identified by Leiper as *Ternidens deminutus*. Leiper also found a single specimen, probably of the same species, in a gorilla from the London Zoological Garden. Brumpt has found specimens in *Macacus sinicus*, and the species has also been reported from *Macacus cynomolgus* in Saigon.

Smith, Fox and White (1908), in America, reported the finding of *Globocephalus macaci* (= probably *T. deminutus*) together with a species of *Oesophagostomum* in the intestine of *Macacus nemestrinus*. One of the two species of parasites occupied submucous cysts, but the authors were unable to state which of the two species was encysted.

Summarising the limited information at present available, it appears that *Ternidens deminutus* is primarily a parasite of Primates (Catarrhinae) which probably serve as the reservoir hosts for man. All human infections thus far recorded have been found in negroes from Central East Africa. On the basis of the parasites received by us from Southern Rhodesia, an additional case may be added to the lists. It seems probable from the evidence available that in this locality, the infection is not an uncommon one. There has been a history of confusion of this parasite with *Ancylostoma duodenale* and *Necator americanus*. No case of infection has hitherto been diagnosed in the living host, nor has the parasite been specifically identified at the time of autopsy so as to permit a study of the habits of the parasites and of the pathological changes that they may induce. Further studies to provide information on these points are indicated.

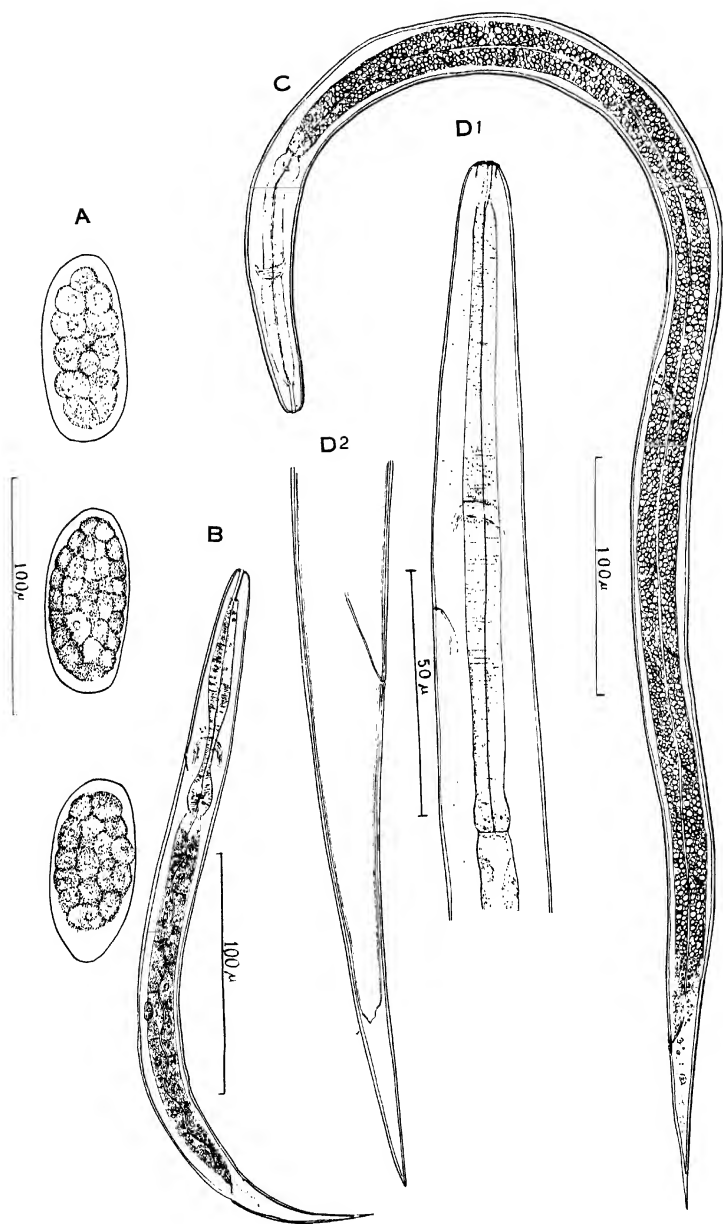
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EXPLANATION OF PLATE II

(All figures were drawn with the aid of the camera lucida. Magnifications are indicated by accompanying scales.)

- A.* Ova found in fresh stools.
- B.* First stage (Rhabditiform) larva.
- C.* Second stage (Rhabditiform) larva in process of transition.
- D1.* Anterior end of third stage (Filariform) larva.
- D2.* Posterior end of third stage (Filariform) larva ensheathed in cuticle of second stage larva.



NOTE ON THE OCCURRENCE OF CRITHIDIA IN *PHLEBOTOMUS* *MINUTUS* VAR. *AFRICANUS* IN NORTHERN NIGERIA

BY

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(Received for publication 20 February, 1929)

Few instances have been recorded of crithidial infections in *Phlebotomus* species. Wenyon (1926) states that flagellates found by Mackie in *P. minutus* in Assam were crithidia, and suggests that the flagellate concerned may represent a trypanosome of a lizard on which these sandflies are known to feed.

Wenyon (1926) also suggests that flagellates found in *P. papatasi*, in Italy, by Laveran and Franchini (1920), and named by them *Herpetomonas phlebotomus*, may be crithidia.

The observations recorded below were made on *Phlebotomus minutus* var. *africanus*, the only species common at Sherifuri. This sandfly is most numerous during the dry season months of April and May, when it attacks man.

It was observed in the course of the wet season of 1928 that *P. minutus* var. *africanus*, although rarely seen elsewhere, was present in large numbers in a cage containing monitor lizards (*V. exanthematicus* and *V. niloticus*). The sandflies could be seen daily feeding on these lizards and also on a small python in an adjoining cage. Although these cages were on the verandah of the laboratory, sandflies were never seen in the building, and apparently were feeding entirely on reptiles.

Examination of the blood content of the mid-gut in 155 of these sandflies containing recognisable blood, showed that over 99 per cent. of the blood was reptilian. A reptilian haemogregarine was common in many of the guts examined.

During August and September, 1928, 304 *Phlebotomus* (301 ♀♀, 3 ♂♂) were caught in the lizard cage and dissected. In 31 ♀♀,

flagellates were found in the mid-gut, giving an infection rate of 10.2 per cent. In all the infected sandflies examined, the infections were confined to the mid- and hind-gut.

Stained smears of the infected guts proved the infections to consist entirely of *T. grayi* type crithidia, their morphology corresponding with the description of *T. grayi* crithidia given by Minchin (1908).



FIG 1. Flagellates in gut of *Phlebotomus minutus* after feeding on *Varanus*. $\times 1200$.

Light infections consisted only of the short, broad type of crithidia, while heavier infections contained also long slender forms. In no case were trypanosomal or spherical forms seen.

The heaviest infections were found in sandflies in which digestion was far advanced (two to three days after feeding). Sandflies containing fresh blood, and those in which digestion was complete, were negative. Between 1923 and 1928, the bloods of 106 *Varanus* of both species have been examined at Sherifuri, and 77, or 68.6 per cent. have been found to be infected with *T. varani*. Lloyd, Johnson, Young and Morrison (1924) showed that *G. tachinoides* fed on infected *V. exanthematicus* developed a flagellate infection of the mid-gut, which was indistinguishable from crithidia of *T. grayi*.

Six of the *Varanus*, which were in the cage from which sandflies were obtained for dissection, were subsequently killed, and in four of them the blood was found to contain trypanosomes. The python,

which was a possible alternative source of infection, was killed, and its blood proved on examination to be negative.

The flagellates in *P. minutus* var. *africanus* described above were evidently crithidial forms of *T. varani* undergoing incomplete development in the sandfly ; it is possible that their occurrence is accidental.

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THE RELATIONSHIP BETWEEN ECONOMIC DEVELOPMENT AND RHODESIAN SLEEPING SICKNESS IN TANGANYIKA TERRITORY

BY

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(Received for publication 25 February, 1929)

WITH MAP

Before the War there was very little Rhodesian Sleeping Sickness known in what is now Tanganyika Territory, and what was known was confined to the Southern part of the country. Since the War three large outbreaks have come to light, one in Mwanza, South-east of Lake Victoria, one in Ufipa and Tabora, and one in Liwali. The last appears to have been a local epidemic superimposed on endemic conditions (Dye, 1927) while, in the case of the other two, years of enquiry have failed to elicit any information about previous endemic foci.

It may not be possible at this stage to clear up definitely the atmosphere of conflicting theories that surrounds the genesis of these outbreaks, but it may be of some interest to endeavour to trace some of the changes that have taken place in tsetse distribution in comparatively recent years. (To appreciate the effects of the changes to be described it is necessary to bear in mind that the type of open country such as is met with in Central Mwanza, all the Districts of Tabora, and numerous other places would revert to tsetse forest if that forest were not kept at bay by human activity. Abandon cultivation at the forest edge and, unless possibly very intense grazing prevents it, the forest will rapidly spread over the abandoned ground, and generally, tsetse infestation will follow.)

In an enquiry such as this one naturally turns first to records of early travellers, but these records, unfortunately, are lacking in the type of information required. For instance, Stanley, on his way to find Livingstone, passed through a large open forest which extends, with few breaks, for tens of thousands of square miles and

(now at least) largely infested by *Glossina morsitans*, between Tabora and Ujiji. Both these travellers returned through the same forest, and again Livingstone when he set out on his last journey passed, in a different direction, through the same forest ; but though we meet

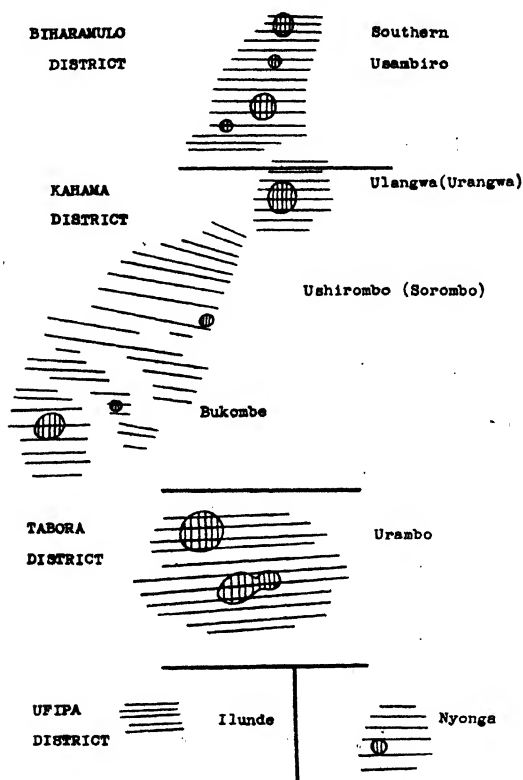


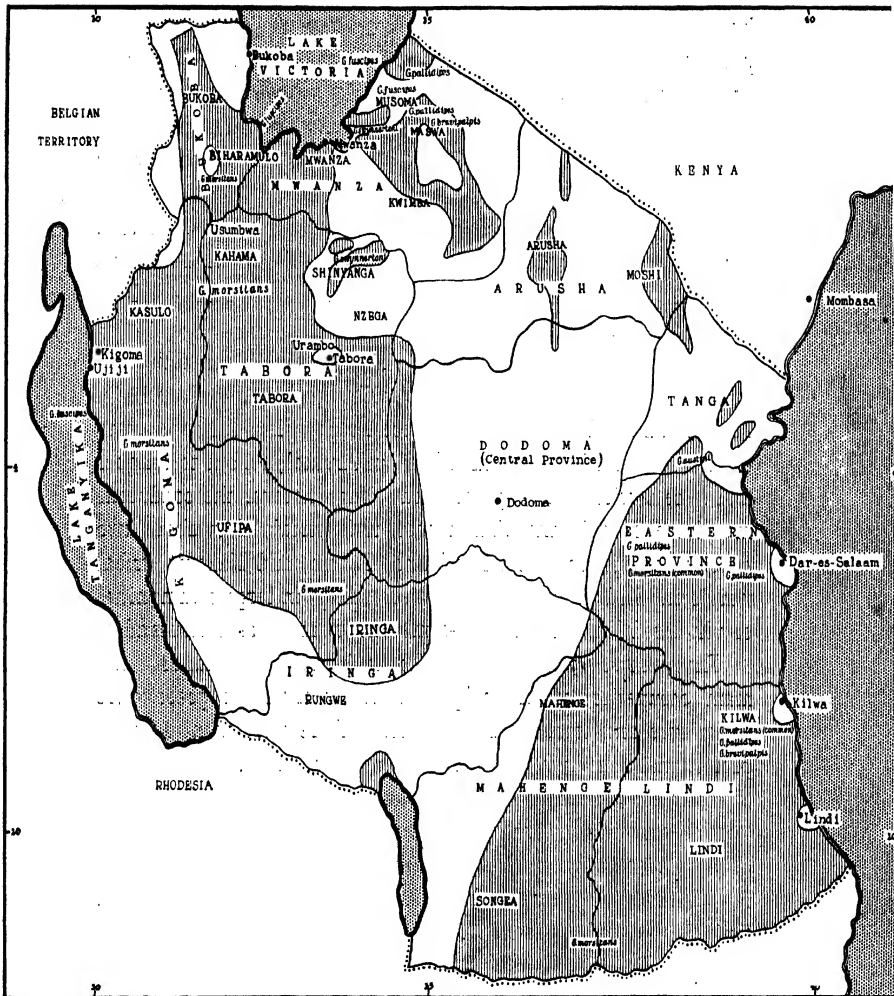
DIAGRAM TO ILLUSTRATE REVERSION FROM OPEN COUNTRY TO TSETSE BUSH
IN DIFFERENT DISTRICTS.

===== = Country once open reverted to bush by 1925.

= Country still open.

Scale (rough estimate only) : 1 inch = 4 miles.

with a reference or two in the latter's journal which places tsetse within perhaps 50 miles of Tabora town on the South, there is nothing to indicate that this was its boundary, or what, in the infested part, was the everyday contact between man and fly.



MAP OF TANGANYIKA TERRITORY.

Rhodesia =

Tsetse Distribution (small clearings in the
general forest not shown) = |||||

Provincial Boundaries =

Provinces = T A B O R A.

Districts = TABORA.

Towns = ● Tabora.

Chiefdoms = Urambo.

Scale : 1 = 5,000,000

After the Veterinary Dept. Tsetse Map, slightly modified.

Stanley's record of his journey from Uganda to Ujiji, in 1876, is of interest in that, though he does not mention tsetse, he gives the population of several towns, among them the capitals of Usambiro and Ushirombo (Sorombo), with 2,000 and 5,000 people respectively. (Only a few people remain in these now and both are surrounded by young tsetse infested forest, while the second of them is now in the midst of Sleeping Sickness area.)

What information is lacking in such records, however, is to a limited extent supplied by natives, and this information can frequently be confirmed by observing the state of the trees and land in different parts of the forest.

Information of more than two generations back is generally very vague. It would appear that some tribes, like the Wakamba (in Mwanza and Kahama), reclaimed forest lands when they wished to extend ; while others, like the Masai (in Arusha), set out to conquer and seize territory already occupied ; but whatever the conditions may have been in the distant past, it is certain that about two generations ago, in places like Ufipa, Tabora, and Kahama, tribal wars compelled the people to live, for safety, in large communities of a hundred families or more—sometimes as many as a thousand.

Round these settlements were the grazing and arable lands. The main occupation of the people in the intervals of peace were agriculture and stock-raising. Whether there were then, as now, professional fishermen who spent a large part of the dry season at rivers infested with *G. morsitans* and *G. swynnertoni*, is not clear, but it seems unlikely that the unsettled state of the country would allow it to be as extensive as it is now. The bee industry which now flourishes appears to have existed then only for local and limited needs. Most communities had professional hunters. The older people who remember those days are emphatic that isolated family villages did not and could not exist then. Even when a raid took place and was successful it rarely if ever resulted in the building of small remote villages.

The usual result was that the sacked settlement was wiped out, the survivors being taken as slaves or escaping to take refuge with some friendly neighbour.

We thus find that in the days before European domination the forest peoples lived in clearings where houses, wells and a large

section of the arable land must have been almost or completely tsetse free all the year round. While there were hunters and, possibly also, honey-collectors and fishermen, the normal life must have been such as reduced the contact between man and fly—even assuming that tsetse was as numerous in the surrounding forests as it is now—to a minimum.

From time to time during seasons of tranquillity a 'daughter' community was formed. An area of virgin or semi-virgin forest was chosen, the trees felled and a new settlement started. This was no individual effort but an organised affair under the leadership of some scion of the chief's family. The new settlement was thus a large one from the beginning.

With the coming of European administration this state of affairs underwent a gradual change. Punitive expeditions played their part in the early days in upsetting the old regime, but this was only temporary and its effects were comparable to the results of the old tribal wars. What was much more far-reaching and permanent in its effects was the establishment of peaceful administration. One of the earliest results was seen in the chiefdoms possessing large numbers of slaves (e.g., Urambo, Usumbwa, etc.). Such of these as had recently been taken from their homes and now liberated by the German Government, grasped their opportunity and returned to their own country. This was a serious matter for such chiefdoms as depended on their slaves to till the land.

The next important result was the evolution of a new type of forest village. With the suppression of tribal wars the necessity for living in large villages for protection no longer existed. There was now an opportunity for benefiting by law and order without having to shoulder the responsibilities of citizenship and many natives took it. It was now possible for a man and his family to go into the forest and start a remote settlement on their own. The virgin soil yielded ample crops and they were comparatively free from the control both of their chief and of the new administration.

The rise of this type of family village in the midst of tsetse has been one of the most important predisposing causes in the epidemiology of Rhodesian Sleeping Sickness.

This disintegration of the large communities, which commenced more than a generation ago, is still going on. Its progress would

probably have been more rapid but for the fact that cattle labour, are less ready to take part in it than others. When they do so they usually leave their stock behind so long as it is healthy for them to remain.

The next aspect of the situation was the demand for labour. The country had to be developed as well as administered and on the heels of the administrator came the trader and the planter. Houses had to be built and roads and railways had to be made. Labour was also required for the sisal and other plantations that were now growing up.

In certain areas only a fraction of the labourers recruited for these purposes returned to their old homes. They settled elsewhere, sometimes taking their own womenfolk with them. This drain on the population contributed still further towards the breaking up of the large forest communities.

Finally the late War gave revolutionary force to the disintegrating changes that were taking place. The exigencies of the times made it necessary to commandeer porters, cattle, sheep, goats, etc., and many communities date their tsetse problem from the time that the army of one or other of the combatants passed through. Influenza followed with dire results.

With peace came a certain amount of reconstruction, but two important factors remained. Families still continued to live in small bush villages, enjoying their hunting and general freedom from control, the number of villages increasing as old lands became over-cultivated or unpopular.

People with a more modern bent left their forest homes permanently to settle in the more populous parts of the country and under more civilised conditions.

The demand for labour, at first small, rapidly increased. The forest native, living, as he often does, far from markets and means of transport, has found that labouring on an estate or on railways, docks, etc., is a convenient means of getting his tax, and supplying his general wants while, at the same time, he enjoys the advantages of travel.

The labour of these people, who do actual work for only a portion of the time that they are absent from their homes, is thus largely lost to their chiefdoms; local development is hampered, more land reverts to bush and a vicious circle is established.

The protection afforded by cleared and cultivated or grazed land is lost and at certain times of the year tsetse is found almost everywhere, occasionally even inside the houses. This applies to both *G. morsitans* and *G. swynnertoni*. When this stage is reached all that is necessary is the introduction of Trypanosome infection to break up what homes remain and complete the devastation.

There are some infected places where the situation is not so acute, where for instance people, mainly fishermen, honey collectors, or hunters, whose homes may be in open country, contract infection while following their vocations ; but these are in the minority and cause concern only in so far as they are threatened by the increasing fly-infestation already described.

This, briefly, is the situation as it affects the chief Sleeping Sickness districts, at least so far as Ufipa, Tabora, Kahama, and to a lesser extent, Mwanza are concerned. But there is another and a more hopeful aspect. Just as old forest settlements are dwindling, modern enterprise is reclaiming vast stretches of bush, for plantations, in other parts of the territory, particularly near the coast, and it is largely the people whose lands are being invaded by tsetse that have done the actual work of reclamation for the plantations.

There are at present no accurate data available by which we can calculate our gains and losses, but it is not improbable that at least as much has been gained as lost. One important factor in the situation is the great leakage of man power that is taking place. The permanent homes of the labourers are frequently hundreds of miles from the scenes of work and many months are lost yearly in travelling and loitering. It is possible that in course of time most of the labourers, instead of maintaining their old homes, will take their families with them to live permanently on or in the vicinity of the plantations on which they work. A movement of this nature has already commenced on a small scale, but for various reasons—such as attachment to their own chiefdom, the freedom of forest life, etc.—the movement is not popular and is unlikely to furnish an early solution of the problem. This drift of native activity towards the plantations and the newer industries must continue for many years and while it continues there will be a corresponding change, and a change for the worse unless dealt with, in the tsetse and Sleeping Sickness situation.

The picture which I have endeavoured to draw only depicts that

section of the population which, caught in the tide of modern enterprise, had no local interests strong enough to root them to their old homes.

There is another section, living for the most part in fly-free or almost fly-free country, who are fast developing their local farming resources. Unless this section specialises in stock raising to the neglect of agriculture and thereby allow tsetse to encroach on them, they stand to gain considerably and not to lose by modern progress.

It is the evolution of this type of community and what it stands for in the way of native endeavour that must be aimed at in any campaign against Rhodesian Sleeping Sickness. Successful agriculture is one of the most powerful weapons that can be at present employed against any tsetse of the *Morsitans* group.

In taking stock of our prospects for the future the Sleeping Sickness areas of the territory may be regarded as divided into three ill-defined zones. In the first zone the people can be settled in large communities under conditions similar to those enjoyed by that section of the population just described, by selecting and clearing unoccupied land for them on the borders of the open country. In these new settlements large numbers of the young men may leave their homes and go out to work and see the world, but most of them will come back. What leakage may occur is likely to be made good by natural increase and by immigration from less favourable localities. The measures necessary to ensure successful agriculture in these communities need not be entered into here.

In the other two zones, where, for various reasons, people must still have their homes in the forest, the dangerous family village already described can be eliminated by settling the people in large clearings. It has already been shown in Tanganyika Territory that this can be safely done even in the middle of an epidemic.

In the second of these zones transport difficulties are compensated for by some local advantage such as rich soil, a good honey forest, etc. As in the first zone the people are likely to maintain numerical strength in spite of a certain leakage.

In the third zone the people, situated far from the main markets and having no special local advantages, can only hold their own by accepting a lower standard of living. Generally speaking these settlements are likely to dwindle gradually as the people drift

towards the more favoured parts of the country and, probably, the best that can be done for them is to show them how to keep their homes as free from tsetse as possible and at the same time develop such resources as they possess. In a country whose possibilities both in mineral and farm products are still so imperfectly explored it would probably be a mistake to eliminate by wholesale evacuation even these outlandish settlements, at least, so long as they do not remain a serious reservoir of trypanosome infection.

SUMMARY

Before the days of European domination the people of Tanganyika Territory lived for the most part under conditions which made it difficult for Rhodesian Sleeping Sickness to get a firm footing.

The freedom that followed the coming of the European resulted in the evolution of a type of bush family village which became a danger to health because of the close contact between man and tsetse that it made possible.

Some of the old large settlements shrank to the dimensions of these villages from various causes, the chief of which were the disturbances produced by the Great War and the re-distribution of population that has resulted from the opening up of the country.

The situation can be met by encouraging native agriculture where this can profitably be done. In places so remote that successful development is problematical the people can be collected in settlements large enough to be safe from extinction by Trypanosomiasis. The people in these settlements have a tendency to drift towards places where better conditions prevail. When this takes place on a large scale the dwindling settlements will have to amalgamate for self-protection, a process of gradual and orderly evacuation will have set in, and continue unless some local attractive source of wealth is discovered to turn the tide.

Once the bush village is compulsorily eliminated it should be possible for reclamation and evacuation to go on in an orderly fashion, as the exigencies of the general situation demand. It should also be possible for native and non-native enterprise to go on with no more antagonism than a legitimate rivalry for labour and for markets.

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NOTES ON THE ANATOMY OF *STILESIA HEPATICA*, AND ON THE GENERA OF THE SUB-FAMILY *THYSANOSOMINAE* (INCLUDING *AVITELLININAE*)

BY

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I. ON THE ANATOMY OF *STILESIA HEPATICA*

Amongst the Cestodes in the collection of the Liverpool School of Tropical Medicine there are a large number of specimens from different sources referable to the sub-family *Avitellininae*; apparently the collection contains all the known, together with a number of new, species.

During the examination of this material the writer found one species which at first seemed to present remarkable characteristics in that there appeared to be four paruterine organs in each segment, viz., two on each side, except in the very gravid posterior ones, in which only two paruterine organs occurred, viz., one on each side. A careful examination of the worm left no doubt whatever that the species is *Stilesia hepatica* Wolffhügel, 1903, and the opportunity is here taken of supplementing the excellent accounts of the anatomy of this species given by Stiles and Hassall (1893), and Gough (1911).

The worms were obtained from (1) Liver of sheep, Durban, South Africa; (2) *Cobus cob*, gall bladder and bile ducts, East Shore, Lake Albert, Tonya, Mr. Thwaite; (3) Hepatic duct of goat, Rhodesia, Professor Yorke; and (4) Sheep, South Africa, A. W. Noel Pillers, Esq., F.R.C.V.S.

They attain a length of about 50 cm. and a maximum breadth of about 3 mm. A large number of the anterior segments, i.e., those containing the male genital organs, are extremely shallow and resemble a pile of saucers. They have a length of about 20μ and a breadth of about 2 mm.; those posterior segments which contain the fully-developed paruterine organs have a length of about 320μ and a breadth of 1.2 mm. The posterior margin of one segment

overlaps the succeeding segment, as shown in figs. 5, 6, and 9, thus enabling one to determine, without any uncertainty, the important point as to which margin is anterior and which is posterior. The genital pores are irregularly alternate and are situated near the middle of the lateral margin of the segment. The pore leads into a rather long and narrow genital atrium, at the base of which the male and female genital ducts open. The cirrus sac is invariably anterior and ventral to the vulva on both sides of the strobilus (fig. 1).

The longitudinal muscles, according to Gough,

‘are in two layers, an inner consisting of bundles of about 12, the outer of 3 or 4 muscles, the transverse muscle is weak as is also the dorso-ventral muscle.’

Figure 2 is a *camera lucida* drawing of a transverse section of the male mature part of the worm. It will be seen that the longitudinal muscles are in one layer only. The bundles are all very very small, each consisting of from about two to twelve fibres; no trace of a sub-cuticula layer of longitudinal fibres could be found in transverse sections from different parts of the strobilus. In our specimens the circular muscular layer is well developed.

The male genital organs have been fully and accurately described by him, and need not be further considered.

He remarks that,

‘There is but one ovarium, lying on the pore side, between the dorsal and ventral canals, nearer to the ventral than to the dorsal canal.’

He figures it as situated amongst the testes. An examination of a large quantity of material, including whole mounts, transverse, longitudinal and horizontal sections, leaves no doubt that Gough's statement is correct. In transverse sections it is seen situated ventrally, between the ventral and dorsal excretory vessels (fig. 2). Directly dorsal to it is the lateral poral dilatation of the transverse uterus, whilst on the aporal side a similar dilatation develops. From the base of the genital atrium the vagina first dilates into a vulva which lies dorsal to the cirrus sac. Near the median extremity of the latter organ the vagina narrows and pursues a slightly sinuous course almost to the level of the dorsal excretory vessel, where it bifurcates, one branch going to the more ventrally situated ovary (which quickly atrophies), and the other branch to the lateral poral dilated termination of the uterus, which, as noted above, is situated immediately dorsal to the ovary (fig. 2). Although both Stiles

and Hassall, and Gough state that a receptaculum seminis is present, in our specimens the vagina nowhere dilates, i.e., a definite receptaculum seminis is absent. Gough points out that,

'The uterus is double, one uterus lying close to the ovary, the other on the other side of the proglottid in the corresponding position. The two uteri are connected by a duct, the inter-uterine duct, which, however, may be morphologically but the median portion of the uterus.'

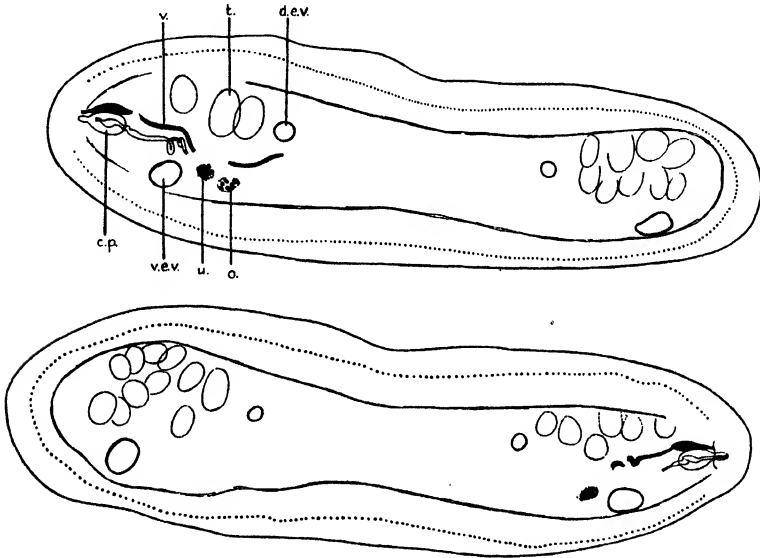


FIG. 1

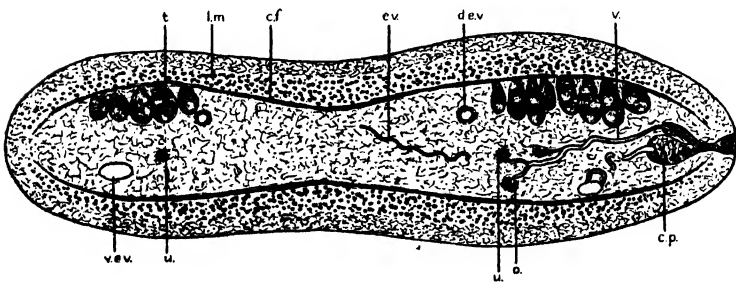


FIG. 2

The uterus is, therefore, in reality single, roughly dumb-bell shaped, extending across the segment, with one lateral dilated extremity dorsal and close to the ovary, and the other lateral dilated extremity

on the opposite side of the segment, the two being for a short time connected by a narrow tube which runs across the segment. Stiles refers to eggs which

'travel across the segment and enter the uterus on the other side at first no ova are seen in either uterus, then they appear in the uterus on the pore side, and at the same time a broken line of ova can be seen extending across the segment toward the opposite uterus; the latter is next observed containing ova, while no ova can be distinguished in the median field. I am forced to admit that in no one segment has it been possible to find a continuous line of ova extending across the entire median field, but by combining several segments a diagrammatic line of ova may be constructed which extends from ovary [*sic* T.S.] to uterus. An interesting point in connection with this wandering of the ova across the segment is that the young ova, especially those found in the median field, have no definite form, a fact which points to their being capable of amoebic motion.'

Gough, in his figures of *S. hepatica* and *S. globipunctata*, shows the duct, and in his account states that it exists, but he does not describe it.

The duct was very clearly seen in our sections (figs. 3 and 4) but it should be noted that it is an extremely delicate and transient

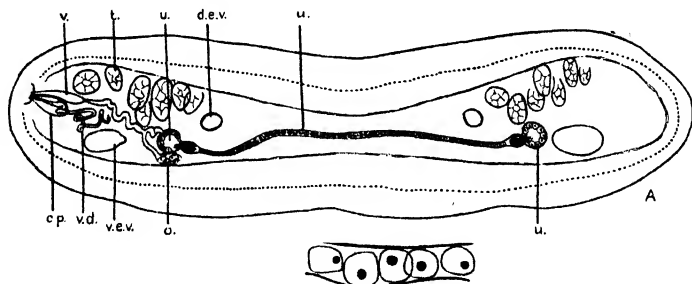


FIG. 3

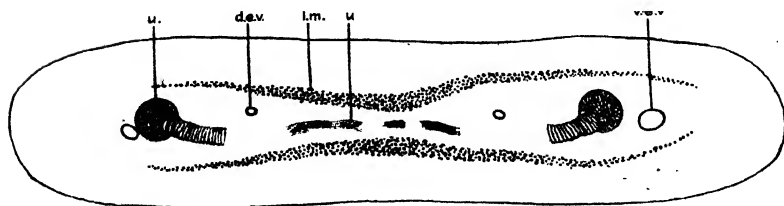


FIG. 4

structure, having a diameter of about 16μ , only found in a limited number of segments, about twenty, which are in a particular stage of development, viz., in those in which the lateral uterine dilatations are

developed, and before the appearance of the paruterine organ. In segments at this stage the tube is well defined and full of eggs, which latter pass across in single file. The duct, however, quickly atrophies, as shown in fig. 4, and the lateral globular dilatations of the uterine tube, one on each side, do *not*, at any future stage in their development, communicate with each other.

The paruterine organ begins to develop within the dilated lateral extremity of the uterus on each side, almost as soon as the uterine cavity appears. In the meantime there appears anteriorly, and somewhat median to it, an almost solid, globular organ, consisting of fibrous tissue arranged in concentric layers which I shall refer to as the paruterine pouch (figs. 5, 6 and 7). The two organs, on one side are in communication with each other by a narrow aperture, as shown in fig. 8. The concentric lamellar fibres from the paruterine pouch invade the cavity of the paruterine organ and eventually become arranged in the form of elongated columns; it is into these columns that the eggs pass. During this development the paruterine organ gradually becomes less and less conspicuous, whilst the paruterine pouch becomes increasingly prominent and large; so that in whole mounts, and in sections, there are to be seen two globular organs on each side of each segment, situated between the dorsal and the ventral excretory vessels. When the paruterine pouch is *fully* developed the lateral dilatations of the uterus (i.e., the paruterine organs) have partly atrophied and are indistinct, the result being that in each segment there appears to be only one globular organ (paruterine pouch) on each side of the segment. In some segments, the lateral dilatation of the uterus (paruterine organ) on one side of the segment, partly atrophies before the lateral dilatation of the uterus (paruterine organ) on the other side of the segment. Consequently, in these segments, under low magnifications, three globular organs only are to be seen, two on one side and one on the other; so that in segments situated a little distance from the posterior extremity, there are in each segment, two globular organs, on each side (one being the lateral dilatation of the uterus containing the paruterine organ, the other being the paruterine pouch) and they are approximately of the same size. In the most posterior gravid segments only one globular organ on each side of the segment is seen, viz., the paruterine pouch.

Segments between these two extremes show that the lateral dilatation of the uterus containing the paruterine organ gradually gets less and less and apparently finally disappears.

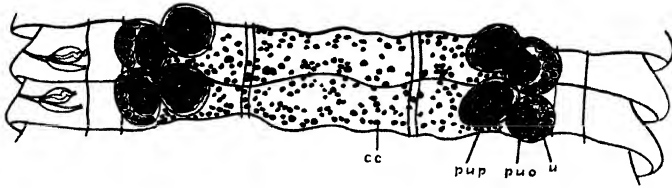


FIG. 5

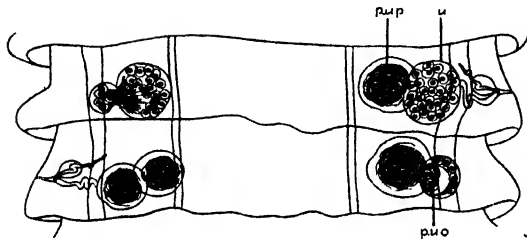


FIG. 6

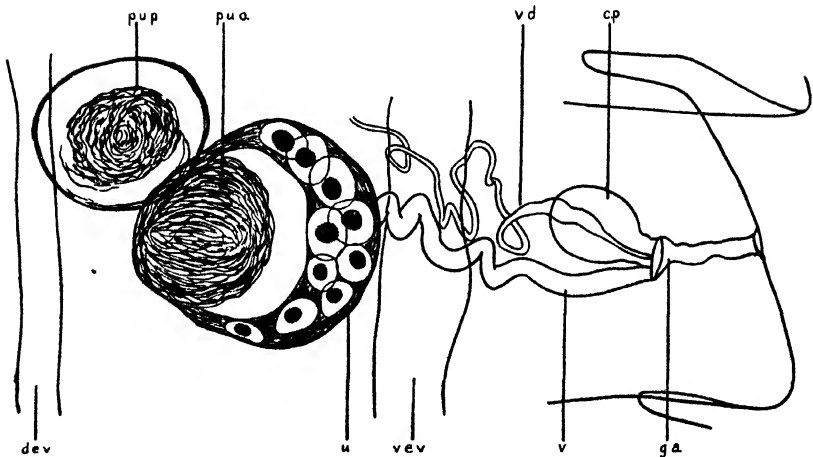


FIG. 7

Stiles and Hassall, Gough, and Baer figure in the fully gravid segments a very wide and prominent area full of granules, occupying almost all the middle of the segment, gradually tapering laterally on each side to a bluntly-pointed cap-like termination which rests on the

anterior part of the paruterine pouch. A structure similar to what these authors figure is often extremely conspicuous in the posterior part of the strobilus, but it is not present in all strobila, whilst in other strobila it is present in some gravid segments and absent in others. On account of its position and appearance, especially in whole mounts, it might easily be mistaken for a uterus full of eggs.

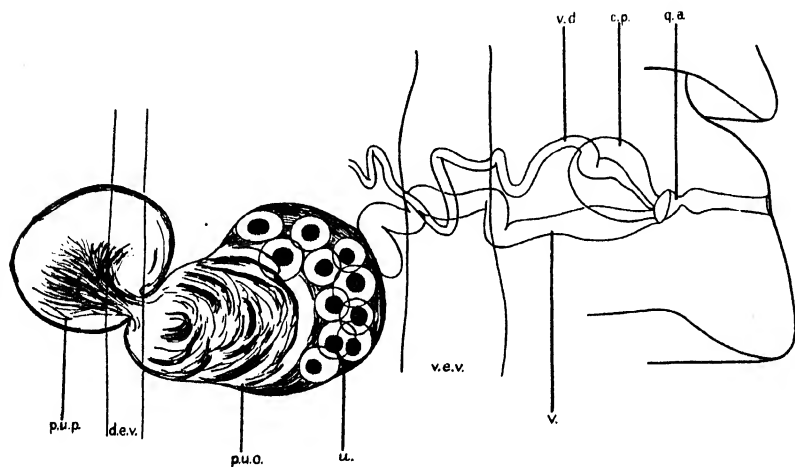


FIG. 8

Having regard to the fact that, as we have seen above, the narrow, median uterine tube which runs between the dilated uterine terminations on each side quickly atrophies, the writer was unable to understand the nature of the structure figured by the above authors, and indicated also in my fig. 9. Careful investigation, however, revealed the following facts :—

1. The granulations only occur in partly or wholly gravid segments, and they are not quite, but almost exclusively limited in distribution to the medullary parenchyma.
2. Even in the most gravid segments the granulations vary in size from about 3μ to 25μ ; they show no cellular structure and have a somewhat irregular outline.
3. They are not contained in a sac but lie free in the parenchyma and are not in communication on either side with the uterus or paruterine organ.
4. As noted above they are frequently entirely absent in some

gravid strobila ; in other strobila they are present in some segments and absent in others.

Stiles states that,

'In many segments one sees at the posterior edge, especially in the lateral fields, a row of small (4 to 8 μ) round or oval bodies which stain very dark in haematoxylin or carmine. These same bodies are occasionally met with in the median field, but their presence is extremely irregular. In general appearance they resemble, to a certain extent, the calcareous bodies found in *T. crassicollis*, *T. solium*, etc.'

The writer is of opinion that these granulations are probably calcareous corpuscles which develop extensively in the increasingly gravid segments.

The eggs produced by each segment of this and, in fact, by all species of this genus, are few, usually about thirty, and are limited in position to the uterus and paruterine organ on each side.

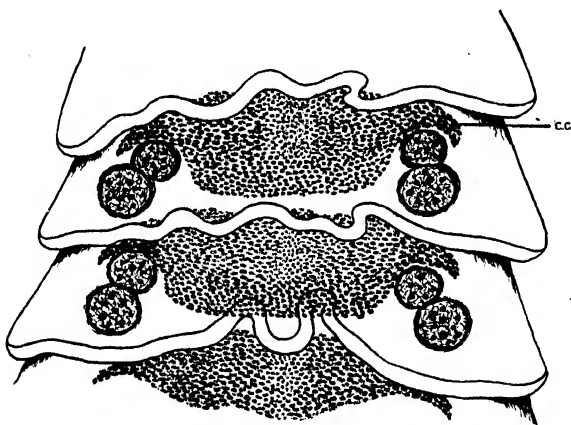


FIG. 9

II. ON THE GENERA OF THE SUB-FAMILY THYSANOSOMINAE (INCLUDING AVITELLININAE)

In 1874, Rivolta described two worms from sheep in Italy, viz., *Taenia globipunctata* and *T. centripunctata*.

Railliet, in 1893, erected the genus *Stilesia* to accommodate the two species described by Rivolta, which clearly could not be retained in the genus *Taenia*. The type-species is *S. globipunctata*. He also described a new species, viz., *S. vittata*, from the camel.

In the same year Stiles revised the diagnosis of the genus as follows :—

‘ Head with four suckers, but no hooks ; strobila broader than long. Two distinct sets of testicles present in each segment, one on each side, but no testicles in the median line. Eggs very small and with one shell. . . . The following points, which may prove to be of generic value, have been established only for *S. globipunctata* ; genital canals pass dorsally to the nerve and ventral canal, but ventrally of dorsal canal. Egg-shell with two conical projections at opposite poles.’

In 1903, Wolffhügel obtained another species (*S. hepatica*) from the bile ducts of sheep and goats in South and East Africa. The worm was described as double-pored.

In 1907, Fuhrmann described a fifth species which, he stated, was also double-pored, viz., *S. sjöstedti*, and at the same time he also placed the genera *Stilesia* and *Thysanosoma* in a new sub-family which he named *Thysanosominae*.

Ransom (1909) had doubts as to whether the genus *Stilesia* should not be included, along with the other genera possessing paruterine organs, in the sub-family *Paruterininae*, even though species referred to the latter sub-family are, for the most part, parasitic in birds.

Gough (1911) pointed out that all species which at that time were included in the genus *Stilesia* possessed neither vitelline gland nor shell gland. He also drew attention to the fact that none of the species had double pores, and that *S. centripunctata* differed from the other species in having four distinct sets of testes and a single uterus in each segment, the other species possessing only two sets of testes and a double (? T.S.) uterus in each segment. He accordingly erected a new genus which he named *Avitellina*, to accommodate *A. centripunctata*. The two genera *Stilesia* and *Avitellina* were placed in a new sub-family which he named *Avitellininae*. His definition of the sub-family and of the two genera was as follows :—

‘ *Avitellininae* : Scolex without hooks with four suckers, segments short, genital pores irregularly alternating. Testicles in two or four groups, marginal, none in the middle field. A single ovary, no vitelline gland, no shell gland ; uterus single or double, eggs finally enclosed in a paruterine organ. Eggs in ovary and uterus surrounded (and nourished) by nutritive cells. Oncosphere with two envelopes.

Type-genus : *Avitellina* Gough, 1911. All known species inhabit ruminants ; development unknown.

Stilesia Railliet, 1893. Type-species : *Stilesia globipunctata* (Rivolta), Railliet, 1893.

Head with four suckers, but without hooks. Strobila thin and narrow. Genital pores irregularly alternate. Segments broader than long. Two distinct sets of testicles present in each segment, one on each side, but no testicles in the median line. Ovarium on the pore side. No vitelline gland, no shell gland. Uterus double, finally void of eggs, which are contained in egg pouches (paruterine organ). The genital canals pass dorsally of the nerve and the ventral canal, and ventrally of the dorsal canal. Eggs with two envelopes. Habitat : Intestine of sheep, goat and dromedary, and bile ducts of sheep, goat, and South African wild antelopes (Africa, India, Italy and France).'

We have, however, noted above that the uterus in this genus is single.

'*Avitellina*, nov. gen. Type species : *Avitellina centripunctata* (Rivolta). Head with four suckers, but without hooks. Strobila thin and narrow. Segments broader than long, flat in the proximal portion of the strobila, nearly cylindrical in the posterior portion. Genital pores irregularly alternate. Four distinct sets of testicles in each segment, one right and one left of each longitudinal canal, but no testicles in the middle field. Ovarium nearer the pore side ; no vitelline gland, no shell gland ; a single uterus. Eggs finally enclosed in egg-pouches (paruterine organ). The genital canals pass dorsally of the nerve and longitudinal canals. Eggs with two envelopes. Habitat : Intestine of sheep, Africa, Italy.'

Blei (1921) erected the genus *Hexastichorchis* for a parasite from sheep, with the following characters :—

'Strobila small and relatively thick. Dorsal longitudinal excretory vessel lateral to ventral vessel. Testes in six rows, four after the disappearance of the dorsal vessel. Paruterine organs single. Adults in sheep.

Type-species : *Hexastichorchis pintneri* Blei, 1921.'

There is reason to believe that the species is distinct, and referable to the genus *Avitellina*.

Meggitt (1924) included in the sub-family *Avitellininae* Gough, 1911, the three genera *Stilesia*, *Avitellina* and *Hexastichorchis*. Unfortunately in his key to the genera of *Avitellininae*, Meggitt states that the testes are in two rows, four rows and six rows on each side, in the above genera respectively. This is clearly an error ; the number of testes on each side is half the number given by Meggitt.

Woodland (1927) re-defined the sub-family *Avitellininae* Gough, 1911, and the genus *Avitellina* Gough, 1911, as follows :—

'*Avitellininae* Gough, 1911. Scolex unarmed, with four suckers. Segments very short and the immature segmented strobila exceeding the mature in breadth. Genital pores marginal and irregularly alternating. Testes in two or four columns, marginal, none in the middle field. Ovary is small, compact and amesially placed on the poral side. No separate vitellaria or shell glands. The uterus is single or bi-partite, and develops in its interior a paruterine organ.

Type-genus : *Avitellina* Gough, 1911.

Avitellina Gough, 1911. Strobila thin (save in the gravid region) and narrow. Four columns of testes and a single uterus, with paruterine organ, in each proglottid. Cirrus sacs always shorter than the vulvae in mature segments. Cirrus sacs on right side of strobila usually dorsal to vulvae, and on the left side usually ventral to them. Genital canals pass dorsally to the lateral nerve and the dorsal and ventral excretory canals. Ventral excretory canals in the posterior half of the strobila always very large (the two covering at least one-eighth of the total proglottid breadth).

Type-species : *A. centripunctata* (Rivolta, 1874).'

In 1928, Woodland erected a new genus for a species found in *Taurotragus oryx*, which he placed in the sub-family *Avitellininae*, namely *Anootypus*, with the following characters :—

'Strobila thin (save in the gravid region) and narrow. A single paruterine organ is present in each proglottid. Cirrus sacs equal in length to, or longer than, the vulvae, and constantly situated anterior and dorsal to them. Genital canals pass dorsally to the lateral nerve and the excretory canals. Dorsal excretory canals are absent, and the ventral canals become reduced in size in the gravid region of the strobila. A single layer of longitudinal muscles is present in the parenchyma.

Type-species : *A. edifontaineus* Woodland, 1928.'

A second new species, viz., *A. ricardi* Woodland, 1928, was also referred to this genus.

In the same paper Woodland suggested, in a foot-note, that the definition of the genera *Stilesia* and *Avitellina* might

'be amended by excluding the number of columns of testes as a generic character and by including the presence of both dorsal and ventral canals and a double layer of longitudinal muscles in the parenchyma as additional characters.'

It will be shown later that a double layer of longitudinal muscles only occurs in certain species, and that the dorsal vessel is very variable.

Baer (1927), following Fuhrmann, placed in the sub-family *Thysanosominae* Fuhrmann, 1907, all those genera which had previously been placed in the sub-family *Avitellininae* Gough, 1911. He defined the sub-family *Thysanosominae* as follows :—

'Large worms. Genital pores double or single; in the latter case they are irregularly alternate. Genital canals dorsal to excretory vessels or between them. Testes very numerous or few, in a single field, or in two lateral groups. Female genitalia in poral half of segment. Vitelline gland may be absent in which case the ovary contains the nutritive cells. Uterus tubular, sometimes very long and undulating. Paruterine organs present; may be very numerous or single. They each contain several eggs. Adults in ruminants.

Type-genus : *Thysanosoma* Diesing, 1835.'

The latter genus previously contained three species, viz., *T. actinioides* Diesing, 1835; *T. giardi* (Moniez, 1879), from sheep, cattle, etc., and *T. pygargi* Cholodovsky, 1902, from *Capreolus pygargus*.

Baer, however, limited the characters of the genus *Thysanosoma* so that it contained one species only, viz., *T. actinioides*. He erected two new genera, viz., (1) *Ascotaenia*, to accommodate *T. pygargi*, and (2) *Helictometra*, to accommodate *T. giardi*. He defined the above three genera as follows :—

'*Thysanosoma* Diesing, 1835. Worms of medium size; posterior edges of segments fimbriated. Two sets of genitalia in each segment. Genital canals pass between excretory vessels and dorsal to the nerve. Testes very numerous, occupying the whole posterior half of the segment between the two ovaries. No vitelline or shell gland. Uterus a single transverse tube which becomes undulated and expels its eggs into numerous paruterine organs. Adults in ruminants.

Type-species: *Thysanosoma actinioides* Diesing, 1835.'

'*Ascotaenia* Baer, 1927. Worms of large size with indistinct segmentation. Genital pores irregularly alternate. Genital canals pass dorsal to excretory vessels. Testes situated on each side of the female sexual glands, limited laterally by the excretory vessels. Female genitalia situated in the poral half of the segment. Vitelline gland small. Uterus a transverse tube becoming sac-shaped. There are eight to twelve paruterine organs, each containing several eggs. Adults in mammals.

Type-species: *Ascotaenia pygargi* (Cholodovsky, 1927).'

'*Helictometra* Baer, 1927. Worms of large size. Genital pores irregularly alternate. Genital canals pass between the excretory vessels and dorsal to the nerve. Testes disposed outside the excretory vessels forming two lateral fields. Female genitalia situated in poral half of segment. Vitelline gland and shell gland both rudimentary. Uterus an undulating tube almost filling the entire segment. Paruterine organs very numerous, each containing several eggs. Adults in ruminants.

Type-species: *Helictometra giardi* (Moniez, 1879).

Baer further considers that the genus *Hexastichorchis* is synonymous with the genus *Avitellina* and that the species *H. pintneri* Blei, 1921, is synonymous with *A. centripunctata* (Rivolta, 1874). It is to be noted, however, that in *H. pintneri*, the relative positions of the dorsal and ventral excretory vessels to each other is quite different from that obtaining in any other genus in the sub-family.

The sub-family *Thysanosominae* Fuhrmann, 1907; according to Baer, thus contains the following genera :—

- | | | |
|-------------------------------|--------------------|------------------------------|
| (1) <i>Thysanosoma</i> | with one species. | |
| (2) <i>Avitellina</i> | with two species. | Woodland, in 1927, described |
| = <i>Hexastichorchis</i> | | four other species. |
| (3) <i>Stilesia</i> | with four species. | |
| (4) <i>Ascotaenia</i> | with one species. | |
| (5) <i>Helictometra</i> | with one species. | |
| (6) <i>Anootypus</i> | with two species. | |

It is decidedly unfortunate that authors have utilised different characters in defining the above genera. Thus Gough differentiated the two genera *Stilesia* and *Avitellina* by the fact that in the former

genus the testes are in two rows, the genital canals pass *between* the excretory vessels, and the uterus and the paruterine organs are double in each segment ; whilst in *Avitellina* the testes are in four rows, the genital canals are *dorsal* to the excretory vessels and nerve, and there is a single uterus and paruterine organ in each segment. Both Woodland and Baer differentiate their genera on characters other than the above.

Baer's two genera *Helictometra* and *Ascotaenia* differ from the other genera of the sub-family in having numerous paruterine organs and also in possessing rudimentary vitelline and shell glands. The two genera differ from each other in the following points :—

In *Ascotaenia* the genital canals pass *dorsal* to the excretory vessels ; the testes are situated on each side of the ovary but internal to the excretory vessels, whilst in *Helictometra* the genital canals pass *between* the excretory vessels and dorsal to the nerve, and the testes are in two lateral rows outside the excretory vessels.

The principal characters attributed to the genus *Anootypus* Woodland, 1928, are :—

1. The cirrus sacs are anterior and dorsal to the vulvae.
2. The genital canals are dorsal to the nerve and excretory vessels.
3. There is a single layer of longitudinal muscles in the parenchyma.

It will thus be seen that the three workers in this group, Gough, Baer and Woodland, base their classifications on different sets of organs and different combinations of characters.

Certain anatomical details may now be considered.

1. *Relation of the cirrus sac to the vulva.* Woodland regards 'the arrangement of the cirrus sacs relative to the vulvae as one of the most important, and so far as we know, constant characters distinguishing *Avitellina* from *Stilesia* (at least equal in taxonomic importance to the number of the paruterine organs.'

It will be seen, however, that this character is less constant than is supposed.

The cirrus sacs in *Anootypus* are said to be anterior and dorsal to the vulvae ; they are definitely anterior and ventral in the case of *S. hepatica*.* Gough finds that in *S. globipunctata* the cirrus sac is ventral to the vagina ; he does not state whether it is situated anterior or posterior, but he figures the one directly ventral to the

* And in *S. vittata* also.

other. Woodland says that the cirrus sacs are invariably ventral to the vulvae, in the genus *Stilesia*.

In *Avitellina*, Gough states that the cirrus sac lies ventral or dorsal, anterior or posterior to the vagina. He gives figures which 'show the sagittal section through about nine sections, passing through four cirri and vaginae; it will be seen that the utmost irregularity has been realised.'

Woodland attaches little importance to this observation and remarks that,

'in all *Avitellina* species the cirrus sacs on the left side of the strobilus lie ventral to the vulvae, and on the right side dorsal.'

In the same paper, he, however, qualifies this statement by saying that the above arrangement obtains in the *majority of cases*. He further asserts that in *A. centripunctata* the sacs are always anterior to the vulvae but only in mature proglottides. The position of the vulvae with reference to the cirrus sacs is not known in the genus *Hexastichorchis*. The relation of these genital ducts to each other in the genus *Avitellina* clearly requires further investigation before a definite conclusion can be reached.

2. *Longitudinal Muscles*. Gough states that in *Stilesia hepatica* and *S. vittata* the longitudinal muscles are in two layers. He makes no reference to the musculature in *S. globipunctata*. From the account given above of the muscular system of *S. hepatica*, it will be noted that the longitudinal muscles in this species are in one layer, not two, and the same is the case in *S. vittata*. Woodland states that in *A. centripunctata* the longitudinal muscles are in two layers, viz., a small sub-cuticula layer and a large layer in the cortical parenchyma. No mention is made of the musculature in his species, *A. lahorea*, *A. sudanea* and *A. chalmersi*, but in his figures of these species the longitudinal muscles are shown in two groups. In the two species of the genus *Anootypus* the longitudinal muscles are in a single layer, as in *S. hepatica*.

3. *Excretory Vessels*. In *Stilesia* and *Avitellina* the large ventral vessel is lateral to the small dorsal vessel, but in *Avitellina* the dorsal vessel is often microscopic and in *A. centripunctata*, a worm measuring up to 285 cm. in length, the lumen of the dorsal vessel is almost obliterated at 40 cm. from the scolex. In *Anootypus* the dorsal vessel is entirely absent, a condition not very different from that obtaining in *A. centripunctata*. It is curious and important to

note that in *Hexastichorchis* Blei, 1921, the large ventral vessel lies *internal* to the small dorsal vessel. This fact is of some importance and it means that *Hexastichorchis pintneri* is, at least, a valid species.

In *Stilesia*, the genital ducts pass between the excretory vessels but dorsal to the nerve, whilst in *Avitellina* they pass dorsal to both the longitudinal excretory vessels and nerve. In *Anootypus* the dorsal excretory vessel is absent and the genital ducts pass dorsal to the ventral excretory vessel and nerve. In certain new species, shortly to be described, the genital ducts appear to run ventral to the excretory vessels.

4. *Testes*. In *Stilesia* the testes are in two rows. In *Hexastichorchis* they are said to be anteriorly in six rows, but the outer row consists merely of one or two irregular testes present only in some segments, and the testes are really in four rows. In *Avitellina* they are also in four rows, but in *A. lahorea* Woodland, 1927, and *A. sudanea* Woodland, 1927, the outer column of testes is only one testis deep and they are absent in some segments, whilst in other segments more than one testis may be found. In *Anootypus* they are said to be in either two or four rows.

5. *Uterus*. This organ is single in *Avitellina*, *Anootypus* and *Hexastichorchis*; Gough states that it is double in *Stilesia*, but as we have seen elsewhere, both he and Stiles note that the two uteri are connected by a duct, the inter-uterine duct which they are inclined to consider as the median portion of the uterus. I have shown above that it is single in each segment, and consists at first of a transverse tube having a dilated lateral termination on each side of the segment, and that later on the transverse tube atrophies, leaving the dilated lateral extremities isolated.

6. *Paruterine Organ*. In *Avitellina*, *Anootypus* and *Hexastichorchis* this organ is single; in *Stilesia* it is double. In a new species to be described shortly the organ is single and *lateral*. Gough writes:

'It is a question I cannot attempt to decide, whether the paruterine organ of *Stilesia* and *Avitellina* is homologous to the paruterine organ of other cestodes, as where it has been observed previously it has generally been held to arise outside the uterus. I am retaining the name as being convenient and as referring to a more or less well-known structure but without prejudice as to its origin in other species. The paruterine organs of various cestodes may quite possibly be of different origin, and may only be convergent structures, as Fuhrmann has shown that they can arise independently in various unrelated genera.'

The following table summarises the principal characters of four of the genera in question :—

	<i>Stilesia.</i>	<i>Avitellina.</i>	<i>Hexastichoborchis.</i>	<i>Anootypus.</i>
Longitudinal muscles.	In a single layer.	In two layers, viz., one small sub-cuticula, the other large in cortex.	In a single layer.	A double layer in neck region only.
Excretory vessels.	Ventral vessel large and <i>external</i> to small dorsal vessel.	Ventral vessel large and <i>external</i> to microscopic dorsal vessel, which latter often atrophies in anterior one-seventh of strobilus.	Ventral vessel large and <i>internal</i> to small dorsal vessel, which latter atrophies near middle of worm.	Dorsal vessel absent.
Cirrus sacs.	Ventral to vulvae on both sides.	Ventral or dorsal, or anterior or posterior to vulvae (Gough). Woodland states that the sacs are always anterior to vulvae; on right side dorsal, on left side ventral.	Relation to vulvae not known, but figured ventral to vulvae.	Always anterior and dorsal to vulvae on both sides.
Testes.	Two rows.	Four rows except in some species where the outer row is absent in isolated segments.	In four rows; but before dorsal vessel atrophies occasionally a single testis lies external to it, giving false appearance of six rows.	One species with four rows, the other species with two rows.
Genital canals.	Between excretory vessels and dorsal to the nerve.	Dorsal to excretory vessels and nerve.	?	Dorsal to excretory vessels and nerve.
Uterus.	Single.	Single.	Single.	Single.
Paruterine organs.	Double.	Single.	Single.	Single.

There appears to be no possibility of securing uniformity in deciding which characters are of specific and which are of generic value, but to the writer it seems desirable to keep separate the genus *Stilesia* mainly on the grounds that the paruterine organs are double,

whilst in *Avitellina*, *Anootypus* and *Hexastichorchis* they are single. It has been noted above that Woodland considers that the relation of the cirrus sacs to the vulvae is a point at least equal in taxonomic importance to the number of paruterine organs. This is not quite true and the desirability of utilising paruterine organs as a basis of classification will be evident when it is realised that these organs are easily seen under low magnifications, and sometimes even with the naked eye, whilst on the other hand the position of the cirrus sac with respect to the vagina, can only be determined after a large number of stained transverse sections have been examined ; and even then, as pointed out above, the relation of these organs to each other appears to vary in at least some species. Further, in species in which external segmentation is indistinct or absent, it is often extremely difficult to determine in the absence of a head, which is anterior and which is posterior. I do not know which of the characters dealt with above are of greatest taxonomic value, either singly, or in combination ; doubtless, opinions on this subject will differ, but it is clear that paruterine organs provide a simple, easy and efficient means of diagnosing the genera *Avitellina* and *Stilesia*.

From what has been stated above it will be obvious that unfortunately four genera, viz., *Avitellina*, *Hexastichorchis*, *Stilesia* and *Anootypus*, have been erected to accommodate about twelve species of cestodes which are closely related and which differ from each other in minor details only—details which appear to the writer, for the most part, of specific value only. I therefore consider that the genera *Anootypus* and *Hexastichorchis* are synonyms of *Avitellina*, and suggest that the characters of the latter genus be modified to accommodate Woodland's two species of *Anootypus*, viz., that the genus *Avitellina* be considered as embracing all those species with a single paruterine organ in each segment ; the testes being in two or four rows.

I accordingly re-define the genera as follows :—

Stilesia Railliet, 1893.

Strobila thin and narrow ; outer segmentation apparently always distinct. Longitudinal muscles always in a single layer in the cortex. A single set of genital organs in each segment. Testes in two rows. Cirrus sacs ventral and usually anterior to the vulvae on both sides. Genital ducts pass between the excretory vessels and dorsal to the

nerve. Uterus single, but paruterine organs double, in each segment. Parasitic in ruminants.

Type-species :—*S. globipunctata* (Rivolta, 1874).

Avitellina Gough, 1911.

SYNONYMS :—*Hexastichorchis* Blei, 1921.

Anootypus Woodland, 1928.

Strobila thin and narrow ; outer segmentation either distinct or indistinct. Longitudinal muscles in a single layer in the cortex, a second smaller layer of sub-cuticula fibres may also be present. A single set of genital organs in each segment. Testes in two or four rows. Cirrus sacs dorsal or ventral and either anterior or posterior to the vulvae. Genital ducts dorsal to both excretory vessels when two are present. Uterus and paruterine organ single in each segment. Parasitic in ruminants.

Type-species :—*A. centripunctata* (Rivolta, 1874).

We have noted above that Baer has re-united the two sub-families *Thysanosominae* and *Avitellininae* into one sub-family for which he retains the name *Thysanosominae* and which he has accordingly re-defined. The following table summarises the characters ascribed by Baer to the two sub-families *Anoplocephalinae* and *Thysanosominae*, into which, together with the sub-family *Linstowinae*, he divides the family *Anoplocephalidae*.

	<i>Anoplocephalinae.</i>	<i>Thysanosominae.</i>
Genital pores	Double, unilateral, irregularly alternate or absent.	Double or single, in latter case irregularly alternate.
Genital ducts	Dorsal to both excretory vessels, except in one genus where they pass between them.	Dorsal to both excretory vessels, or between them.
Testes	Numerous, sometimes only two ; in a single field.	Numerous or few ; in a single field ; not in two lateral groups.
Ovary	In poral half of segment.	In poral half of segment.
Vitelline and shell glands ...	Present.	Present or absent.
Uterus	Tubular, sac-like, or reticular.	Tubular.
Paruterine organ	Absent.	Present.

It will be noted that the only essential point of difference between these sub-families is the presence of a paruterine organ in the sub-

family *Thysanosominae*. I agree with Baer in including in this sub-family the following genera, the characters of which may be summarised thus:—

- 1 and 2. *Stilesia* and *Avitellina*, the characters of which I have re-described above.
3. *Thysanosoma* differentiated from all other genera of this sub-family by the possession of a double set of genital organs in each segment ; in addition numerous paruterine organs are present.
4. *Ascotaenia* differentiated from other genera in the sub-family by the fact that the testes are situated between the excretory vessels and do not extend lateral to them ; further, they lie on each side of the ovary, the latter organ being in the poral half of the segment. A vitelline gland is present but is very small. As in *Thysanosoma*, paruterine organs are numerous but it differs from this genus in having a single set of genital organs in each segment.
5. *Helictometra*. In its general morphology this genus resembles *Stilesia* and *Avitellina*. The genital organs are single in each segment and the pores irregularly alternate. The genital ducts pass between the excretory vessels and dorsal to the nerve. Unlike *Stilesia* and *Avitellina*, however, the testes in this genus are situated lateral to the excretory vessels. It also differs from the latter genera in possessing rudimentary vitelline and shell glands and very numerous paruterine organs.

The following key will serve to emphasise the outstanding points of difference between the genera included in the sub-family:—

With a double set of genital organs in each segment ...	<i>Thysanosoma</i>
With a single set of genital organs in each segment ...	1
1. With one paruterine organ in each segment	<i>Avitellina</i>
With more than one paruterine organ in each segment	2
2. With two paruterine organs in each segment	<i>Stilesia</i>
With numerous paruterine organs in each segment	3
3. Testes within the excretory vessels	<i>Ascotaenia</i>
Testes in two fields, one lateral to the excretory vessels on each side	<i>Helictometra</i>

As indicated elsewhere, a further contribution will follow shortly, and will deal with species of certain of the above genera.

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EXPLANATION OF LETTERING.

<i>c.c.</i> = calcareous corpuscles.	<i>p.u.o.</i> = paruterine organ.
<i>c.f.</i> = circular fibres.	<i>p.u.p.</i> = paruterine pouch.
<i>c.p.</i> = cirrus pouch.	<i>t.</i> = testes.
<i>d.e.v.</i> = dorsal excretory vessel.	<i>u.</i> = uterus.
<i>e.v.</i> = excretory vessel.	<i>v.</i> = vagina.
<i>g.a.</i> = genital atrium.	<i>v.d.</i> = vas deferens.
<i>l.m.</i> = longitudinal muscles.	<i>v.e.v.</i> = ventral excretory vessel.
<i>o.</i> = ovary.	

EXPLANATION OF FIGURES.

Stilesia hepatica.

- FIG. 1. Outline of the transverse sections of two succeeding segments showing the relationship of the vulva and the cirrus pouch. $\times 53$.
- FIG. 2. Transverse section of mature segment showing the musculature and male and female genitalia. $\times 53$.
- FIG. 3. *A.*—Outline of transverse section of mature segment showing transient uterus. $\times 53$.
B.—Uterine tube more highly magnified. $\times 400$.
- FIG. 4. Outline of transverse section of mature segment showing atrophy of the transient uterine tube.
- FIG. 5. Gravid contracted segments showing uterus, paruterine organ, paruterine pouch and (?) calcareous corpuscles. $\times 46$.
- FIG. 6. Gravid segments showing relationship of paruterine organ and paruterine pouch. $\times 46$.
- FIG. 7. Poral side of segment showing paruterine organ and pouch. $\times 240$.
- FIG. 8. Poral side of segment showing paruterine organ and pouch in communication. $\times 240$.
- FIG. 9. Gravid segments showing (?) calcareous corpuscles. $\times 53$.

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THE DISTRIBUTION OF BLACKWATER FEVER IN AFRICA*

BY

J. W. W. STEPHENS

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TWO MAPS

ABYSSINIA.

Locality	Cases	Authority
<i>Diré-Daoua</i>	1 (1911)	Pichoy (1912), p. 617.

ALGERIA.

Locality	Cases	Authority
<i>General</i>	<p>Parrot (1915) states that almost all the foci are situated in maritime or juxta-maritime regions, at the mouths of rivers or in the valleys they drain—(1) Seybouse and Saf-saf in the East, Macta in the West; (2) the high plateaux; foci at Aïn-touta, Batna Tiaret; (3) oases of the Sahara; (4) sporadic cases at Algiers, Alma (Mitidja), Guyotville.</p> <p>' Nous pouvons évaluer à la centaine la totalité des cas connus, pour la période qui va de 1899 à la fin de 1920.'</p> <p>' La fièvre bilieuse hémoglobinurique tendait à prendre une place importante dans la pathologie des Européens d'Algérie.'</p> <p>' Paraît extrêmement rare en 1912.'</p>	<p>Parrot (1915), p. 29.</p> <p>Parrot (1921).</p> <p><i>loc. cit.</i>, p. 59.</p> <p>Campagne (1913), p. 71.</p>

* Stephens, J. W. W. (1927). The distribution of blackwater fever in Europe. *Ann. Trop. Med. & Parasitol.*, **21**, 467.

—— (1928). The distribution of blackwater fever in South West Asia. *loc. cit.*, **22**, 53.

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—— (1928). The distribution of blackwater fever in Burma and the Far East. *loc. cit.*, **22**, 179.

ALGERIA—continued.

Locality	Cases	Authority
Département d'Alger—		
<i>Alger</i>	1 (1906)	Sergeant, Ed. and Et. (1907), p. 32.
<i>Alma</i> (<i>Plain of La Mitidja</i>)	1 (1910) 1 (1914)	Parrot (1915), p. 29.
<i>Bouira</i>	'Localité réputée depuis longtemps comme très paludéenne et où l'on constate presque chaque année des cas de fièvre hématurique.'	Sergeant, Ed. and Et. (1921), p. 330.
<i>Guyotville</i>	1 ()	Parrot (1915), p. 28.
<i>Maison-Carrée</i> (<i>near Algiers</i>)	1 (1901)	Brault (1903), p. 561.
Département de Constantine—		
<i>Ain-touta (Mac-mabon)</i> ...	'Des cas de fièvre bilieuse hémoglobinurique y sont constatés chaque année.'	Anon (1923), p. 473.
	... (1917)	Parrot (1921), p. 60.
	... (1918)	
	1 (1919)	
	... (1920)	
	(Native) 1 (1921)	Parrot (1923), p. 607.
	4 (1917-21)	<i>loc. cit.</i>
<i>Barral Guebar</i>	2 (1905)	Sergeant, Ed. and Et. (1911), p. 146.
<i>Batna</i>	'On y constate chaque été des accès de fièvre à forme pernicieuse et des cas très graves parfois mortels, de fièvre bilieuse hémoglobinurique.'	Anon. (1923), p. 432.
	'Les cas d'accès pernicieux et de bilieuse hémoglobinurique sont fréquents dans ces "quartiers".'	Ballet (1923), p. 556.
	4 (1918)	Sergeant, Ed. and Et. (1921), p. 330.
	5 (1913)	Campagne (1914), p. 25.
<i>Duzerville</i>	1 (1912)	Parrot (1915), p. 29.

* ... Under the column 'Cases', signify that cases are recorded but that the number is not stated. This does not apply to the tables with eight columns.

ALGERIA—continued.

Locality	Cases	Authority
Département de Constantine—(contd.)—		
<i>El'-Arrouch</i>	4 (1899-1908) 2 (1909) 7 (1910) Several (1911) o (1912)	Parrot (1915), p. 29.
<i>Gutbar</i>	Sergeant, Ed. Correspondence.
<i>Guelma</i>	Parrot (1915), p. 28.
<i>Mondovi</i> (Adjacent Localities)	6 (1901-10) 1 (1905) Several (1906) (1907) (1908) (1909) 1 (1910) 3 (1911) 2 (1912) 2 (1910)	Parrot (1915), p. 29. Sergeant, Ed. and Et. (1911), p. 146. <i>idem</i> (1914), p. 77.
<i>Morris</i>	1 (1908) 1 (1920)	Parrot (1915), p. 29. Sergeant, Ed. and Et. (1921), p. 331.
<i>Ouled-Rabmoun</i>	1 (1903)	Sergeant, Ed. and Et. (1904), p. 324.
<i>Pentbièvre</i>	2 (1910)	Sergeant, Ed. and Et. (1911), p. 148.
<i>Robertville</i>	'Cas nombreux.'	<i>idem</i> , p. 132.
<i>Robertville</i>	6 deaths (1909) 7 „ ? (1910) 3 „ ? (1911) o (1913) o (1914) o (1915) 36 (18 years) 4 (1908-11) 32 (1911-27)	Ciavaldini (1917). Ciavaldini (1927).
<i>Seybouse, Valley of</i>	4 ()	Sergeant, Ed. and Et. (1911), p. 96.
<i>Taber...</i>	2 (1921)	Anon (1923), p. 485.
<i>Touggourt</i>	1 (1920)	Sergeant, Ed. and Et. (1926), p. 331.
Département d'Oran—		
<i>Cbeliff Valley</i>	Sergeant, Ed. Correspondence.
<i>Habra Plain</i>	
<i>Macta Valley</i>	

ALGERIA—continued.

Locality	Cases	Authority
Plain of the Macta— Arzew to Ain-Tedeles		
<i>Arzew</i>	25 (1904-05)	Coste (1906).
<i>Cassaigne</i>	Parrot (1915), p. 28
<i>Palikao</i>	Campagne (1914), p. 52. Sergeant, Ed. and Et. (1915), p. 5.
<i>Schdou</i>	4 (1904)	Claude (1905), p. 274.
<i>Tiaret</i>	Parrot (1915), p. 28.
<i>Tourville</i>	Sergeant, Ed. Correspondence.

ANGOLA.

Locality	Cases	Authority
<i>General</i>	8 deaths	Da Silva (1898, 1899). Plehn (1899), p. 240.
<i>Benguela</i>	3 (1905) 1 (1910)	Pinheiro (1906), p. 276. Barreto (1913), p. 107.
<i>Loanda</i>	'Malaria in a great number of cases took on a grave form, blackwater fever, and per- nicious.'	Pinheiro (1906).
<i>Mossamedes</i>		Barreto (1913).
<i>Moçambique</i>		
<i>S.S. Beira</i>		

BASUTOLAND.

Europeans	Cases	Deaths	Natives	Cases	Deaths	Year	Authority
1603 (year 1921)	No records.	...	495,937 natives 1,241 Coloured	No records	...	1926	Basutoland (1927).

BECHUANALAND.

Locality	Cases	Authority
	1 (1927-28)	Bechuanaland Protectorate, p. 12.

CAMEROONS.

Europeans	Cases	Deaths	Natives	Cases	Deaths	Year	Authority
...	24	1903-04	Medizinal, pp. 150, 159.
826	30	5	1904-05	pp. 94, 112, 113.
896 + 85	31	9	...	1 (Syrian)	0	1905-06	pp. 143, 155, 162.
Military	19	1	1906-07	pp. 101, 110, 121, 127.
	32	4	1907-08	pp. 221, 229, 237.
...	31	4	...	1	1	1908-09	pp. 172, 177, 180, 199, 245.
...	38	6	1909-10	pp. 250, 255, 258, 381.
...	28	2	...	1	...	1910-11	p. 467.
...	1911-12	
...	30	1911-13	Steudel (1924), p. 33.
Cameroon (French)	8	3	1923	Letonturier (1924), p. 396.
(Douala Hospital)	'La plupart de ces malades, colons anciens ou surmenés, avait contracté leur affection en dehors de Douala.'						

CONGO.

Locality	Cases	Authority
Mid-Congo State ...	'I have had considerable experience among patients suffering from this disease—over a hundred—all of whom recovered.'	Banks (1900), p. 111.
Congo ...	50 (or 40) 20 negroes from the Antilles, 20 Europeans engaged on railway construction.	Plehn (1899), p. 239.
Stanley Pool ...	25	loc. cit.
Basin of the Congo ...	18	Védy (1907).
Brazzaville ...	2 (1922)	Blanchard and Lefrou (1922), p. 699.
	4	Ringenbach (1915), p. 120.
Leopoldville ...	16 (1899-00)	Campenhout and Dryepontd (1901), p. 55.
	12 (1900-01)	Broden (1906), pp. 8, 58.
	25 (1902-05)	loc. cit., p. 8.
	20	Houssiau (1919).
	10	Van Hoof (1924)

DAHOMY.

Locality	Cases	Authority
	<p>'Sur les cinq Européens qui ont été atteints de cette maladie en 1921, un ne prenait jamais la moindre dose de quinine.'</p> <p>'Gouzien . . . pendant son séjour à Dahomey a traité . . . 53 cas.'</p>	Gautier (1922).

ERITREA.

Locality	Cases	Authority
<i>Gasc</i>	'I have been informed that a few very rare cases have been found in Eritrea in the region of Gasc and Setit.'	Professor Franchini (1929). Correspondence.
<i>Setit</i>		

FRENCH EQUATORIAL AFRICA.

Locality	Cases	Authority
<i>Loango</i>	7 (1887-1888)	Gros (1890), p. 47.

GABON (FRENCH EQUATORIAL AFRICA).

Locality	Cases	Authority
<i>General</i>	'Anderer Ansicht ist Calmette, welcher in Gabun 1886-87 sich sehr häufig mit dem Schwarzwasserfieber zu beschäftigen hatte.'	Mense (1899).

GAMBIA.

Europeans	Cases	Deaths	Natives	Cases	Deaths	Year	Authority
151	3 ^a	1	10,000 (African) 100 (Mixed)	1910	Gambia— pp. 25, 32.
230 ¹	4 ^a	2	7,470	1911	pp. 18, 25.
230 ¹	3 ^a	...	7,470	1912	p. 40.
230 ¹	0	...	7,470	1913	p. 25.
230 ¹	0	...	7,470	1914	p. 39.
...	0	0	1915	
...	1 ^a	1916	p. 24.
...	0	1917	p. 25.
...	0	1918	p. 16.
...	2 ^a	1919	p. 13.
...	3 ^a	1920	p. 13.
238	2 ^a	1921	pp. 5, 17.
205	1 ^a	1	9,395	1922	p. 28.
210	6 ^a	4	9,567	1923	p. 34.
218	5	2	9,741	1924	pp. 6, 7, 8, 26.
...	2	1	...	1	1	1925	pp. 16, 17.
...	5 ^a	2	1926	pp. 22, 45.
...	17	3	1927	pp. 5, 22, 32, 46.

(1) Apparently refers only to Bathurst (Gambia).

(2) Apparently all European cases.

GAMBIA—continued.

Locality	Cases	Authority
<i>MacCarthy Island (Gambia)</i> (About 126 miles up the River Gambia)	1 (1910)	Gambia, p. 37.
	1 (1914)	p. 39.
	1 (1917)	p. 26.

GOLD COAST.¹

Europeans	Cases	Deaths	Natives	Cases	Deaths	Year	Authority
436	2	2	...	1893	Gold Coast (1894)— pp. 25, 26.
769	12 ²	5	...	1	1	1895	(1897), pp. 12, 14, 15, 24.
798	1896	(1897), pp. 12, 21.
...	1897	
...	1898	
...	4	2	1899	(1909), p. 16.
...	5	1900	<i>loc. cit.</i>
...	8	1901	<i>loc. cit.</i>
1,830	9	4	1902	<i>loc. cit.</i> , pp. 7, 16.
1,796	8	6	1903	<i>loc. cit.</i>
1,953	15	3	1904	<i>loc. cit.</i>
1,911	19	2	1905	<i>loc. cit.</i>
1,765	22	3	1906	<i>loc. cit.</i>
1,877	27	4	1907	<i>loc. cit.</i>
1,768	21	7	1908	(1909), p. 43.
1,715	17	3	1909	(1910), p. 6.
1,692	20	5	1910	(1911), pp. 9, 11, 39.
2,245	8	2	1911	(1912), pp. 7, 9, 39.
2,367	14	6	1912	(1913), pp. 10, 13.
2,590	21	5	1913	(1914), pp. 11, 13, 56, 62.
2,645	21	5	1914	(1915), pp. 13, 15, 75.
2,006	9	2	1915	(1916), pp. 10, 11, 31, 35.
2,001	16	3	1916	(1917), pp. 9, 10, 23.
2,172	24	8	1917	pp. 8, 10, 30.
1,823	17	4	1918	pp. 21, 40.
3,182	14	7	...	6	1	1919	(1919), pp. 7, 8, 10.
2,818	36	7	1920	(1921), pp. 8, 34.
2,939	21	9	1921	(1922), pp. 9, 32.
2,901	48	6	1922-23	(1923), pp. 8, 61.
3,043	21	7	1923-24	pp. 8, 46.
3,104	16	2	1924-25	pp. 7, 38.
3,104	13	5	...	5 ³	2	1925-26	pp. 7, 10, 34.
3,481	7	3	1926-27	pp. 14, 105.

(1) The Gold Coast includes Gold Coast Colony, Ashanti and Northern Territories.

(2) Whether non-official European cases have been recorded is not evident. The 12 cases include 3 deaths—haematuric fever (1), haemorrhagic fever (1), and haemoglobinuric fever (1).

(3) 2 African.

FRENCH GUINEA.

Locality	Cases	Authority
<i>Conakry</i>	1 (1895) 'De cette observation et des nombreux cas analogues... à Conakry.'	Maclaud (1895).
	9	Le Moal (1905).
	6 (1920)	Pelletier and Quemener (1921).
	8 (1904)	Le Moal (1907), p. 258.
<i>Boke</i>	14 (1904-05)	<i>loc. cit.</i>
<i>Conakry</i>	
<i>Dakar</i>	
<i>Conakry</i>	73 (Aug., 1900-Mar., 1904)	Pinard and Boyé (1904), p. 493.
<i>Railway</i>	12 (Aug., 1900-Mar., 1904)	
<i>Ballay Hospital</i>	16 (1899) 14 (1900) 8 (1901) 10 (1902) 14 (1903) — 62	
<i>Railway</i>	2 (1910)	Savignac (1911), p. 474.
<i>Siguiré (Haut Niger)</i> ...	6	Quennec (1895).
	1	Quennec (1899).

IVORY COAST.

Locality	Cases	Authority
<i>Grand Bassam</i> 2 (1895) 6 (1911-13)	Crosse (1899), p. 120. Hébrard (1895). Sorel (1913).
<i>Haut Sassandra, Daloa and Soubré</i>	'La fièvre bilieuse hémoglobulinurique a été observée à Daloa et à Soubré, aussi bien au début du séjour qu'après plusieurs années de présence à la colonie mais toujours chez des impaludés.'	Blanchard (1911).
<i>Abidjan</i>	4 Europeans	Vivie (1907).
<i>Bassam</i>	13 "	
<i>Labou</i>	2 "	
<i>Toumodi</i>	7 "	
	— 26 (1905)	

KENYA (formerly East Africa Protectorate).

Europeans	Cases	Deaths	Natives including Asiatics	Cases	Deaths	Year	Authority
3,656	1911	East Africa Protectorate.
4,913	14	1912	p. 52.
6,713	5	2	...	10	3	1913	pp. 28, 31, 118, 121.
7,297	11	3	...	9	1	1914	pp. 74, 77.
...	15	4	...	4	1	1915	pp. 74, 77.
...	16	1	...	4	1	1916	pp. 64, 67.
...	3	0	...	15	5	1907	pp. 74, 77.
...	2	1	...	16	5	1918	pp. 71, 75, 76.
...	29	8	...	11	4	1919	pp. 82, 83.
...	4	2	...	9	2	1920	Kenya, pp. 92, 93.
9,651	12	1	...	16	4	1921	pp. 128, 129.
...	18	3	...	21	7	1922	pp. 120, 121.
...	8	2	...	20	4	1923	pp. 84, 85, 86.
...	8	4	...	13	5	1924	pp. 66, 71.
...	15	5	...	35	10	1925	pp. 86, 96.
...	21	6	...	34	10	1926	pp. 72, 77, 82, 84.
...	17	3	...	18	5	1927	pp. 52, 57, 62, 67, 72.

KENYA—continued.

Locality	Cases	Locality	Cases	Authority
1913-1927				
<i>Eldama Ravine</i> ...	2	<i>Kakamega</i> ...	3	East Africa Protectorate. Kenya.
<i>Eldoret</i> ...	14	<i>Kilindini</i> ...	10	
<i>Embu</i> ...	1	<i>Kisii</i> ...	3	
<i>Fort Hall</i> ...	15	<i>Kismayu</i> ...	2	
<i>Kacheliba</i> ...	5	<i>Kisumu</i> ...	65	

KENYA—continued.

Locality	Cases	Locality	Cases	Authority
<i>Kitui</i>	6	<i>Mumias</i>	5	Kenya
<i>Lamu</i>	3	<i>Nairobi</i>	96	
<i>Lodwar</i>	5	<i>Nakuru</i>	13	
<i>Macbako</i>	3	<i>Narok</i>	13	
<i>Mwerib</i>	3	<i>Northern Takana</i> ...	2	
<i>Makindu</i>	5	<i>Nyeri</i>	2	
<i>Malindi</i>	3	<i>Serenli</i>	3	
<i>Meru</i>	3	<i>Voi</i>	16	
<i>Mombasa</i>	116	<i>Yante</i>	1	
<i>Mueressi</i>	5			
			413	

These figures could only be used for comparative purposes if the population (European) of the various localities were known.

LIBYA.

Locality	Cases	Authority
<i>Tripolitania and Cyrenaica</i>	'There are no cases of this disease in the Italian North Africa where malaria is very rare.'	Professor Franchini (1929). Correspondence.

MOROCCO.

Locality	Cases	Authority
<i>Gbarb District</i>	1 (1922)	Vialatte (1922).
<i>Rabat</i>	1 (1925)	Vialatte (1925).

NIGERIA.

Locality	Cases	Authority
<i>Benin</i>	17	Giraud (1891), p. 406.

NIGERIA, NORTHERN.

Europeans	Cases	Deaths	Natives	Cases	Deaths	Year	Authority
...	21	5	1898	Nigeria, Northern (1907). <i>loc. cit.</i>
...	22	3	1899	
165	12	3	1900	<i>loc. cit.</i>
165	12 ¹	1	1901	<i>loc. cit.</i>
290	20 ¹	5	1902	<i>loc. cit.</i>
309	17 ¹	8	1903	<i>loc. cit.</i>
322	35 ¹	6	1904	<i>loc. cit.</i>
342	18 ¹	4	1905	<i>loc. cit.</i>
347	25 ¹	5	1906	<i>loc. cit.</i>
424	12	0	...	6	1	1907	<i>loc. cit.</i>
499	14	4	1908	Nigeria, Northern.
544	13	3	...	2	0	1909	
637	9	2	...	1	1	1910	
641 ²	12	6	9·27 × 10 ⁶	1	0	1911	
703	14	4	1912	
804	17	4	1913	
969	22	1914	
897	22	4	1915	
762	22	8	...	9	4	1916	
779	19	4	...	2	0	1917	
989	27	7	1918	

(1) The figures include non-Europeans.

(2) Not including 79 Lagos Railway officials.

NIGERIA, SOUTHERN.

Europeans	Cases	Deaths	Natives	Cases	Deaths	Year	Authority
533	...	2	1905	Nigeria, Southern. pp. 143, 150. (1912), pp. 62, 126. (1912). 1911 1912 1913 1914 pp. 42, 76, 80. pp. 8, 46, 51. pp. 12, 21, 45, 50. p. 20.
...	21	4	1906	
...	57	10	1907	
1,244	48	8	1908	
...	29	10	1909	
...	34	7	1910	
1,648	25	8	...	1	1 ?	1911	
...	21	3	...	2	1	1912	
1,589	26	6	8.1 × 10 ⁶	3	0	1913	
...	20	5	1914	
1,650	11	2	...	7	2	1915	
1,650	19	4	...	8	1	1916	
1,650	22	11	...	5	2	1917	
...	29	4	...	1 ¹	0	1918	

(1) African.

NIGERIA.

Europeans	Cases	Deaths	Natives	Cases	Deaths	Year	Authority
...	38	10	...	3	0	1919 ¹	Nigeria (1919-21), p. 73. pp. 7, 64, 70. pp. 6, 49, 55. pp. 7, 12. pp. 12, 43, 49. pp. 45-55. p. 55.
...	33	8	...	6	0	1920	
...	41	10	...	5	1	1921	
...	28	2	...	4	1	1922 ²	
...	25	6	...	11	8	1923	
...	24	12	...	7	0	1924	
4,050	30	7	...	5	0	1925	
4,833	30	8	...	4	1	1926	
5,493	31	5	...	15	3	1927	

(1) Northern and Southern Provinces combined (1919-21).

(2) The returns for the year 1922 and onwards are for the combined Colony and Protectorate of Nigeria, including that portion of the Cameroons now under British mandate.

NYASALAND.

Europeans	Cases	Deaths	Natives	Cases	Deaths	Year	Authority
608	1905-06	Nyasaland. pp. 5, 26, 27. pp. 5, 10, 18. p. 5.
583	1906-07	
587	1907-08	
595	14	3	1908-09	
587	3	3	...	1909-10	
...	5	3	1910-11	
...	4 ¹	1	...	1	1	1911-12	
758 (Europeans and Whites)	10	(Asiatic) 1 (Asiatic)	...	1912-13	
...	6	1	...	1913	
...	3	1	1914	
...	2	1915	(1914), p. 12.
...	2	1	1916	(1915), p. 12.
...	2	1	1917	(1916), p. 10.
...	1	1918	(1917), p. 10.
...	6	1	1919	(1918), p. 9.
...	11	1	1920	(1919), p. 12.
1,655 (Europeans and Whites)	14	2	1921	(1920), p. 11.
...	14	6	1922	(1921), p. 11.
...	13	2	...	1 (Asiatic)	0	1923	(1922), p. 12.
...	5	2	1924	(1923), p. 10.
...	3	3	...	1	...	1925	(1924), p. 9.
...	3	0	1926	p. 8.
...	11	2	...	8	3	1927	p. 7.

(1) 11 in 1912-13 Report.

PORTUGUESE EAST AFRICA.

Locality	Cases	Authority
<i>Angoche</i>	'Mortality : ten times greater than the mortality for malaria.'	Soromenho (1923).
<i>Cbindé</i>		
<i>Inbambane</i>		
<i>Lourenço Marques</i>		
<i>Moçambique</i>		
<i>Quelimane</i>		
<i>Tete</i>	'Blackwater fever is the disease which causes more anxiety than any other to the European population of the whole territory of Moçambique.'	Turner (1910), p. 112.
<i>Moçambique Territory</i>		
<i>Delagoa Bay</i>		
	7	Garin (1910), p. 252.

RHODESIA, NORTHERN.

Europeans	Cases	Deaths	Natives	Cases	Deaths	Year	Authority
4,600	(35) ¹	7	1,140,642 (Africans)	1925	Rhodesia, Northern (1928a), pp. 20, 21. <i>loc. cit.</i>
...	(60) ¹	12	1926	
...	29	9	1927	Rhodesia, Northern (1928b), pp. 13, 39, 42, 45, 48, 74-76.

(1) Cases estimated on a basis of 20 per cent. mortality.

RHODESIA, SOUTHERN.¹

Europeans	Hospital Cases	Deaths	Natives	Cases	Deaths	Year	Authority
...	73	17	...	1	1	1906	Rhodesia, Southern (1908), p. 15. (1908), p. 15. (1909), p. 19. (1910), p. 15. (1911), p. 16. (1912), p. 24. (1913), p. 22. (1914), pp. 31, 34. (1915), pp. 17, 30. (1916), pp. 24, 27. (1919), p. 29. <i>loc. cit.</i> <i>loc. cit.</i>
14,007	57	13	1907	
14,640	41	12	1908	
...	75	18	...	3	1	1909	
...	75	17	...	1	...	1910	
...	39	7	...	1	1	1911	
...	60	17	...	1	...	1912	
...	57	14 ²	...	4	...	1913	
...	53	13 ³	...	0	...	1914	
...	60	16 ⁴	...	2	...	1915	
...	34	5	...	1	1	1916	
...	48	13	1917	
...	32	10	...	4	1	1918	

(1) Southern Rhodesia includes Mashonaland and Matabeleland.

(2) 34, p. 34. (3) European deaths 28, Indians 1, pp. 30, 31. (4) 37, p. 27. Natives 2 deaths, p. 28.

RHODESIA, SOUTHERN—*continued*.

Europeans	Hospital Cases	Deaths	Natives	Cases	Deaths	Year	Authority
38,284	36	7 ¹	...	1	...	1919	Rhodesia, Southern (1920), pp. 29, 32.
...	70	10 ²	...	5	0	1920	(1921), pp. 30, 33, 34.
33,620	53	6 ²	1921	(1922), pp. 7, 39, 40.
...	49	14	...	1	...	1922	(1923), p. 36.
...	64	14 H	}	1923	(1924), p. 19.
...	122	40 T					
...	20	1 H	}	3	2	1924	(1925), pp. 16, 50, 53.
...	39	11 T					
...	52	13 H	}	1925	(1926), pp. 16, 42, 43.
...	78	26 T					
...	37	11 H	}	1926	(1927), pp. 21, 73, 77, 80.
...	...	21 T					
...	36	13 H	}	1	1	1927	(1928), pp. 17, 70, 72, 74, 78.
...	...	15 T					

(1) European deaths 9 (1916), 17 (1917), 17 (1918), 18 (1919).

Native deaths 0 (1916), 0 (1917), 1 (1918), 1 (1919).

The figures for total European deaths from Blackwater do not agree with those given in earlier reports.

(2) Europeans 22 deaths, Natives 2 deaths, pp. 33, 34.

(3) Europeans 23 deaths, Natives 1 death, pp. 39, 43.

H = hospital cases. T = total cases.

SENEGAL.

European cases	Deaths	Native cases	Deaths	Year	Authority
22	5	1905	Merveilleux (1910), p. 693.
44	6	1906	
27	5	1907	
50	8	1	1	1908	
45	12	1909	

SENEGAL—continued.

Locality	Cases	Authority
<i>General</i>	1 (1925)	Huchard (1925).
<i>Bakel</i>	Barthélemy-Benoit (1865).
<i>Dagana</i>	<i>loc. cit.</i>
<i>Dakar</i>	6 (1909)	Rosé (1911).
<i>Dakar</i>	'à Dakar les cas sont fréquents.'	Esquier (1922).
<i>Dakar</i>	
(à l'infirmerie du Marigot)	European and Native Malaria water Strength. Cases. Cases. Year.	
	204 187 0 1911	
	232 247 4 1912	
	291 206 11 1913	
	387 229 1 1914	
	349 291 5 1915	
	363 409 0 1916	
	495 711 1 1917	
	1,103 1,780 11 1918	
	770 846 16 1919	
	4 (1914-16)	Marcandier (1916).
	Cases Deaths Year	
<i>Dakar</i>	12 6 1892-1894	Clarac (1898).
	24 6 1894-1896	
	33 (1909)	Merveilleux (1910), p. 689.
	3 deaths (1926)	Dupont (1928).
<i>Kaslack</i>	Barthélemy-Benoit (1865).
<i>Kéniéba</i>	<i>loc. cit.</i>
(abandonné depuis 1861)	3 (1858-1859)	<i>loc. cit.</i>
<i>Médine</i>	Cases Deaths Year.	
	3 2 1857	
<i>St. Louis</i>	9 4 1858	
(à l'hôpital de)	4 2 1859	
	11 2 1860	
	11 5 1861	
	26 3 1862	
	24 7 1863	
	1 (1911)	Gastou and Dufougerè (1911), p. 301.
	9 (1909)	Merveilleux (1910), p. 689.
	Cases. Deaths. Year.	
<i>Gorée Islands</i>	1 ... (1855)	Barthélemy-Benoit (1865).
	1 ... (1856)	
	2 ... (1857)	
	8 ... (1858)	
	6 ... (1859)	
	4 1 (1860)	
	12 3 (1861)	
	35 10 (1862)	
	39 8 (1863)	
	'depuis vingt ans . . . à Gorée 285 bilieuses hématuriques sur près de 23 mille entrées.'	Béranger Féraud (1874), p. 62.
	2 (1909)	Merveilleux (1910), p. 689.
<i>Saint Louis Island</i>	'depuis vingt ans nous voyons qu'il y a eu à Saint-Louis, 178 fièvres bilieuses mélanuriques seulement, sur près de 43 mille entrées à l'hôpital.'	Béranger Féraud (1874), p. 61.

SIERRA LEONE.

Europeans	Cases	Deaths	Natives	Cases	Deaths	Year	Authority
620	5	1	1909	Sierra Leone (1910), pp. 6, 54, 69.
831 (Europeans and Whites)	12	4	1910	(1911), pp. 6, 24, 49, 60.
...	2	1	1911	(1912), pp. 51, 57.
...	8	4	1912	(1913), pp. 7, 43, 50.
...	9	2	1913	(1914), p. 5.
...	11	4	1914	p. 5.
...	6	0	1915	p. 7.
1,138	3	0	1916	pp. 14, 26.
...	13	1	1917	pp. 13, 14, 33, 38.
1,036	1	1918	pp. 15, 40.
1,176	6	1919	pp. 8, 42.
...	6	1920	p. 38.
...	6	1921	(1922), p. 43.
...	6	3	1922	(1923), p. 39.
...	5	1	...	1	...	1923	(1924), pp. 9, 35, 42.
...	5	1	...	2	...	1924	(1925), pp. 9, 37, 44.
...	3	2	1925	(1926), pp. 6, 34, 41.
...	4	1	...	3	1	1926	(1927), pp. 6, 31, 38.
...	4	2	...	5 ¹	4	1927	(1928), pp. 6, 42, 55.

(1) 1 African.

SOMALILAND.

Locality	Cases	Authority
<i>Protectorate, British</i> ...	'I have seen one patient who seems to have suffered from the disease in Somaliland.'	Crosse (1899), p. 114.
	No records (1923-1927)	Somaliland Protectorate.
	European Population. Natives. Year. 52 ¹ 300,000 1927	
<i>Italian</i>	'One mortal case was reported by one of the doctors of this Institute (Dr. Martinelli) in 1926, in the region of Lugh.'	Professor Franchini (1929). Correspondence.
	'Another doctor of this Institute, Dr. Veneroni, in 1924 reported two cases among the white men in the English Jubaland, which is now an Italian possession (the Oltregiuba).'	
	'La febbre biliosa emoglobinurica nella Somalia Italiana è forse meno rara di quanto si sarebbe indotti a giudicare dalla deficienza di comunicazioni nosografiche e anche da qualche asserzione negativa a riguardo.'	Cosimo (1927).

(1) 72 including Air Force.

SUDAN (ANGLO-EGYPTIAN).

Locality	Cases	Authority
<i>Atbara</i>	1 (1926)	Anon (1928).
<i>Bahr-el-Ghazal</i>	2 (1905) 10 (?)	Ensor (1906).
	'Blackwater fever is certainly more common . . . in the Bahr-el-Ghazal than in some of the districts of the White Nile.'	
<i>Mongalla</i>	1 (1926)	Anon (1928).
<i>Rosieres (Blue Nile)</i>	'A notoriously malarial station where blackwater fever is known to occur.'	Balfour (1913), p. 37.
<i>Wau</i>	3 deaths (1905)	Wenyon (1928) (personal communication).

SUDAN, FRENCH.

Locality	Cases	Authority
<i>Bamako (on the Niger)</i>	Rousseau (1887).
<i>Kati</i>	17	Cardeillac (1905).
	Campagne du Soudan, 1889-1890	Durand (1891), p. 15.
	Cases Deaths	
<i>Bakel</i>	10 ...	
<i>Bammako</i>	4 1	
<i>Faboulabe</i>	3 2	
<i>Kayes</i>	9 2	
<i>Kita</i>	1 1	
<i>Niagassola</i>	1 1	
<i>Siguiri</i>	1 ...	
	<hr/> 29 7 <hr/>	
	<p>' Nous devons donc nous contenter de comprendre dans ce groupe de fièvres bilieuses rémittentes la mélanurique et l'hématurique en faisant toutefois observer que la première est bien plus fréquente que la seconde.'</p>	
<i>Bamako</i>	2 (1896)	Carmouze (1897).
<i>Djenné</i>	1 (1896)	
<i>Kayes</i>	18 (1895-1896)	
<i>Kita</i>	1 (1896)	
<i>Siguiri</i>	1 (1896)	
<i>Tous les postes du Soudan</i> ...	Deaths Year	
	11 (1889)	
	11 (1890)	
	13 (1891)	
	16 (1892)	
	25 (1893)	
	25 (1894)	
	7 (1895)	
	8 (1896)	
<i>Dakol</i>	2	Henric (1898).
<i>Kankan</i>	1	Quennec (1899).
<i>Kita</i>	1 (1899)	

TANGANYIKA TERRITORY (formerly German East Africa).

Locality	Cases	Authority
	23 (1894-95) 32 (1895-96) 19 (1896-97) 30 (1897-98) 32 (1898-99)	Mann (1902).

TANGANYIKA TERRITORY.

Europeans	Cases	Deaths	Natives	Cases	Deaths	Year	Authority
...	30	3	4,107,000	12	4	1921	Tanganyika Territory, p. 11a.
...	44	4	...	3	...	1922	pp. 63, 108.
...	19	4	...	16	3	1923	(1924), p. 138.
...	16	2	...	15	3	1924	p. 138.
...	52 ¹	7	8	1925	pp. 10, 13.
...	85 ¹	10	4,319,000	...	9	1926	(1927), p. 15.
...	72 ¹	6	10	1927	(1928), pp. 14, 19.

(1) Total number of cases. How many European cases is not evident.

Togo (in 1920 divided between Gold Coast and Dahomey).

Europeans	Cases	Deaths	Natives	Cases	Deaths	Year	Authority
...	9	(total)	1901-02	Arbeiten (1904), p. 81.
...	2	(total)	1902-03	<i>loc. cit.</i> , p. 585.
...	1903-04	
...	2	1904-05	Medizinal, p. 117.
...	6	2	1905-06	p. 201.
...	18	3	1906-07	pp. 133, 147.
...	16	2	1907-08	p. 256.
...	6	1	...	2	0	1908-09	p. 314.
...	4	0	1909-10	p. 447.
...	12	0	1910-11	p. 505.

TUNIS.

Locality	Cases	Authority
	... 'Cette maladie n'y a jamais été constatée de façon certaine.'	Gouzien (1911). Nicolle, C. (1929). Correspondence.

UGANDA PROTECTORATE.

Europeans	Cases	Deaths	Natives	Cases	Deaths	Year	Authority
...	10	2	(total)	1904	Uganda Protectorate (1918), p. 12.
...	14	3	(total)	1905	
...	41	4	(total)	1906	
...	10	2	1907	1908 Report type-script.
...	13	2	(majority Asiatics)	1908	1908 Report type-script.
...	21	6	(total)	1909	(1918), p. 12.
...	26	6	(total)	1910	<i>loc. cit.</i>
...	4	1	...	14	2	1911	(1913), p. 10.
...	10	4	2,840,469	35	5	1912	(1913), pp. 10, 17.
823	19	2	2,889,561	39	10	1913	(1914), pp. 12, 19.
1,017	28	8	2,904,454	54	13	1914	(1916), pp. 11, 17.
...	65	18	(total)	1915	(1916), p. 9.
...	46	10	(total)	1916	(1917), p. 8.
...	8	2	...	41	6	1917	(1918), p. 12.
...	40	7	(total)	1918	(1921), p. 43.
894	24	4	...	59	14	1919	<i>loc. cit.</i>
942	12	1	...	44	6	1920	<i>loc. cit.</i> , pp. 11, 44.
...	12	3	...	50 ¹	12	1921	(1922), Appendix.
...	15	4	...	68 ²	10	1922	(1923), Appendix.
...	10	3	...	61 ³	14	1923	Appendix, p. 97.
...	10	2	...	61	21	1924	(1925), Appendix, p. 57.
...	10	3	...	71	19	1925	(1926), Appendix, p. 67.
...	21	2	...	150 ⁴	48	1926	(1927), Appendix, p. 61.
...	20	6	...	86	22	1927	(1928), Appendix, p. 75.

(1) 49 Asiatics, 1 African.

(2) 67 Asiatics, 1 African.

(3) 61 Asiatics.

Europeans 11 cases, 3 deaths. Asiatics 72 cases, 14 deaths. p. 96.

(4) 127 Asiatics.

UNION OF SOUTH AFRICA.

Locality	Cases	Authority
<i>Union of South Africa</i> ...	No records (1927)	Union of South Africa (1927).
1. <i>Cape of Good Hope Province.</i>	Population :—	
2. <i>Province of Natal.</i>	Europeans ... 1,637,472	
3. <i>Province of Orange Free State.</i>	Bantu ... 5,034,563	
4. <i>South West Africa Protectorate (mandated).</i>	Asiatic ... 172,577	
= <i>German South West Africa.</i>	Mixed ... 563,320	
5. <i>Province of Transvaal.</i>	7,407,932	
<i>Cape Province</i> ...	'There is no blackwater fever in Cape Colony.'	Turner (1910), p. 111.
(= <i>Cape Colony</i>)		
<i>Natal</i> ...	'It has, though exceptionally, been seen.'	Clemow (1903), p. 47.
	'There have, however, been two or three cases in the last four years reported as blackwater fever in persons who certainly contracted the disease in Zululand or in Natal, close to the river Tugela.' (Hill.)	Turner (1910), p. 112.
<i>South-West Protectorate (formerly German South West Africa)</i>	11 (1894-95)	Mann (1902).
	5 (1907-08)	Medizinal, pp. 309, 314.
	1 (1910-11)	<i>loc. cit.</i>
	(Europeans ... 10,456)	
	Natives ... 33,344)	
<i>Transvaal*</i> ...	'In the northern portions of the Transvaal such as the Zoutpansberg and Waterberg districts, it is also prevalent.'	Turner (1910), p. 112.
<i>Zoutpansberg District</i> ...	'Since 1896 the cases have followed one another very quickly and I reckon that there are on the average five to ten cases a year in this part of the district where the population is small and much scattered.'	Borle (1911), p. 239.

* *Transvaal and Zululand.*—Dr. W. A. Murray, of Pretoria, in a letter says :—'It is relatively common in the Lowveld of the Transvaal; at Komatiport alone there had been eight or nine cases in 1926.' 'In certain areas of Zululand it is equally common.'

WEST AFRICA.

Locality		Cases	Authority
		'Tableau de la fréquence proportionnelle des maladies endémiques dans les diverses possessions françaises de la côte occidentale d'Afrique (rapporté au dénominateur de 100 hommes et d'un an).	Béranger Féraud (1874), p. 238.
		Fièvre mélanurique.	
		Cases Deaths	
SÉNÉGAL	Gorée	3·03	·91
	St. Louis	0·93	·28
	Moyen	3·90	1·24
	Haut	21·31	4·26
Cayor		7·71	2·33
Rivières de Sud (Cazamance, Rio Nunez)		14·86	3·39
Côte d'Or		37·70	4·01
Gabon		53·05	3·80

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* I have not thought it necessary to give the actual titles of the 'Medical Reports' for British Colonies and Protectorates, for not only do they differ in different places but in many cases they differ from year to year in the same place. In the Sierra Leone 'Medical Reports' we find, for example, the following titles:—

- (a) 'Annual Report on the Medical Department for the Year ended —.'
- (b) 'Annual Report on the Medical and Sanitary Departments for the Year ended —.'
- (c) 'Annual Medical and Sanitary Report for the Year ending —.'
- (d) 'Annual Medical and Sanitary Report for the Year —.'
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ISLANDS

CAPE VERDE ISLANDS.

Locality	Cases	Authority
<i>Saint-Nicolas</i>	'Quant à la fièvre bilieuse hémoglobulinurique affection parapoludéenne, on la rencontre quelques fois à Saint Nicolas (Riberia - Brava) très rarement dans les autres îles.'	Fatome (1907), p. 246.

COMORO ISLANDS.

Locality	Cases	Authority
<i>General</i>	'La bilieuse hématurique s'observe communément.'	Fontoynt (i), pp. 258, 263.
<i>Mayotta</i>	Le Roy de Méricourt (1853).
	'At Mayotta in the Comoro Islands it is very prevalent.'	Clemow (1903), p. 47.
	...	Lafont (1908).
	...	Gouzien (1911).
	'C'est encore chez les créoles que se développe le plus fréquemment la fièvre bilieuse hémoglobulinurique.'	Blin (1905).
	'La fièvre bilieuse hémoglobulinurique présente chez les Européens des caractères très différents.'	
	I	Vaysse (1896), p. 234.
<i>Mohéli</i>	2 (1903)	Lafont (1905), p. 511.
	'La fièvre bilieuse hémoglobulinurique est rare et peut-être faut-il attribuer cette rareté au nombre restreint de blancs. Cependant deux cas suivis de décès sont survenus en 1903. Ce sont les deux premiers cas observés au cours des dix dernières années.'	

CONAKRY.

Locality	Cases	Authority
	<i>vide</i> French Guinea	

FERNANDO PO.

Locality	Cases	Authority
<i>Fernando Po</i>	'Die chinesischen Kulis am Congo und auf Fernando Po haben dagegen sehr schwer unter der Krankheit zu leiden.'	Scheube (1910), p. 70.

GORÉE.

Locality	Cases	Authority
	<i>vide</i> French Guinea	

MACCARTHY ISLAND.

Locality	Cases	Authority
	<i>vide</i> Gambia	

MADAGASCAR.

Locality	Cases	Authority
<i>General</i>	'La bilieuse hémoglobininurique est fréquente dans le Nord-Ouest de Madagascar. Sur une population Européenne très restreinte nous avons eu 10 cas en deux ans et demi.'	Vivie (1903), p. 404.
	'It is common in Madagascar not only in the lower-lying regions but also in the more elevated district of Antsianaka where it prevails especially in the cool season.'	Clemow (1903), p. 47.
	'Il y a des nombreux cas de fièvres rémittentes bilieuses, de bilieuses hémoglobininuriques et d'accès pernicieux.'	Chemin (1904).

MADAGASCAR—continued.

Locality	Cases	Authority																								
<i>General—(contd.)</i>	<p>'En tous les points de l'île se rencontre l'affection; néanmoins le plus grand nombre des cas signalés sur les hauts plateaux sont ceux des gens qui s'étant profondément impaludés dans les régions chaudes de la côte montent dans les régions froides du centre.'</p> <p>65 (1897) 16 (1905) (European troops ... 21,528)</p>	Fontoynt (1897).																								
<i>Tananarive ...</i> <i>L'hôpital</i> <i>d'Isoavinandriana</i>	<p>'Pour le seul régiment d'infanterie représentant un effectif moyen de 1000 hommes . . . 22 décès par suite d'accès pernicieux ou de fièvre bilieuse hémoglobulinurique (1898).'</p>	Salanoue-Ipin (1911), p. 28.																								
<i>Ankadinandriana</i> <i>(native hospital)</i>	<table> <tr> <th>Cases</th><th>Deaths</th><th>Year</th></tr> <tr> <td>1</td><td>1</td><td>(1904)</td></tr> <tr> <td>3</td><td>3</td><td>(1905)</td></tr> <tr> <td>23</td><td>4(?)</td><td>(1906)</td></tr> <tr> <td>34</td><td>7</td><td>(1907)</td></tr> </table> <p>'Ce n'est qu'en 1907 que le diagnostic fièvre bilieuse hémoglobulinurique apparaît sur ces statistiques. Nous relevons pour cette dernière année 41 décès imputables à cette affection, ce qui par comparaison avec le chiffre des hospitalisations et des décès hospitaliers permettrait de supposer environ 200 cas en ville.'</p>	Cases	Deaths	Year	1	1	(1904)	3	3	(1905)	23	4(?)	(1906)	34	7	(1907)	Rigaud (1909).									
Cases	Deaths	Year																								
1	1	(1904)																								
3	3	(1905)																								
23	4(?)	(1906)																								
34	7	(1907)																								
<i>Mananjary and Ranomafara</i>	28 (1921-23)	Celestin (1923), p. 113.																								
<i>Tananarive ...</i> <i>(native hospital)</i>	<p>1 (1904) 3 (1905) 19 (1906) 26 (1907)</p>	Fontoynt (1908), p. 577.																								
<i>Tananarive ...</i>	<table> <tr> <th>Europeans</th><th>Native Troops</th><th>Year</th></tr> <tr> <td>63</td><td>0</td><td>1897</td></tr> <tr> <td>66</td><td>2</td><td>1898</td></tr> <tr> <td>78</td><td>2</td><td>1899</td></tr> <tr> <td>109</td><td>4</td><td>1900</td></tr> <tr> <td>92</td><td>5</td><td>1901</td></tr> <tr> <td>130</td><td>25</td><td>1902</td></tr> <tr> <td>162</td><td>25</td><td>1903</td></tr> </table>	Europeans	Native Troops	Year	63	0	1897	66	2	1898	78	2	1899	109	4	1900	92	5	1901	130	25	1902	162	25	1903	
Europeans	Native Troops	Year																								
63	0	1897																								
66	2	1898																								
78	2	1899																								
109	4	1900																								
92	5	1901																								
130	25	1902																								
162	25	1903																								
<i>Madagascar and Réunion</i>	5 deaths (1897)	Burot and Legrand (1897).																								

MAURITIUS.

Locality	Cases	Authority
<i>General</i>	1 (1908) 'Je pourrais citer maints autres exemples dans lesquels la température a été heureusement influencée par la quinine.'	Senneville (1908).
	28 cases regularly treated without quinine by Dr. de Chazal.	Raffray (1908).
	'Pendant le cours de ma pratique j'ai eu à soigner une vingtaine de cas de fièvre hémoglobinurique.'	Vinson (1908).
	1 (1909)	Chevreau (1909).
	1 (1909)	Raffray (1909).
	No records (1916-1926)	
	Population (1926).	
	Mauritius :	
	General population 111,996	
	Indians ... 277,733	
	Chinese ... 8,507	
	398,236	
	Dependencies ... 9,226	
	407,462	
	13 (1921-1923)	Celestin (1923), 114.
	12 (prior to 1907)	de Chazal (1908), p. 118.
<i>Beau Bassin</i>	2 (prior to 1907)	
<i>Flacq</i>	1 (1907-08)	
<i>Mesnil</i>	1 (1907-08)	
<i>Phoenix</i>	8 (1907-08)	
<i>Port Louis</i>	3 (1907-08)	
<i>Vacac</i>	1 (1907-08)	

Nossi-Bé.

Locality	Cases	Authority
	22	Lebeau (1851).
	...	Le Roy de Méricourt (1853).
	...	Daullé (1857).
	'Ictero-haematuric fever, 185 cases, 49 deaths (1862-1880)'	Davidson (1892).
	2	Yersin (1895).

RÉUNION.

Locality	Cases	Authority.
<i>General</i>	'La bilieuse hématurique au contraire est moins fréquente que dans l'île voisine (Madagascar).'	Fontoy nont (?).
	1 (1873	Monestier (1873).
	Enfin la fièvre hémoglobi- nrique . . . n'offre générale- ment pas ici la sévérité qu'on lui reconnaît à Nossi-Bé.'	Merveilleux (1903).
<i>St.-Denis</i>	'J'ai, en cinq ans et demi observé, chez 45 sujets 54 cas de bilieuse hémoglo- binurique.'	O'Zoux (1911).

St. Louis, *vide* SENEGAL.

St. Marie.

Locality	Cases	Authority
	...	Gouzien (1911).

St. Thomé.

Locality	Cases	Authority
	...	Da Costa (1906).

ZANZIBAR AND PEMBA ISLANDS.

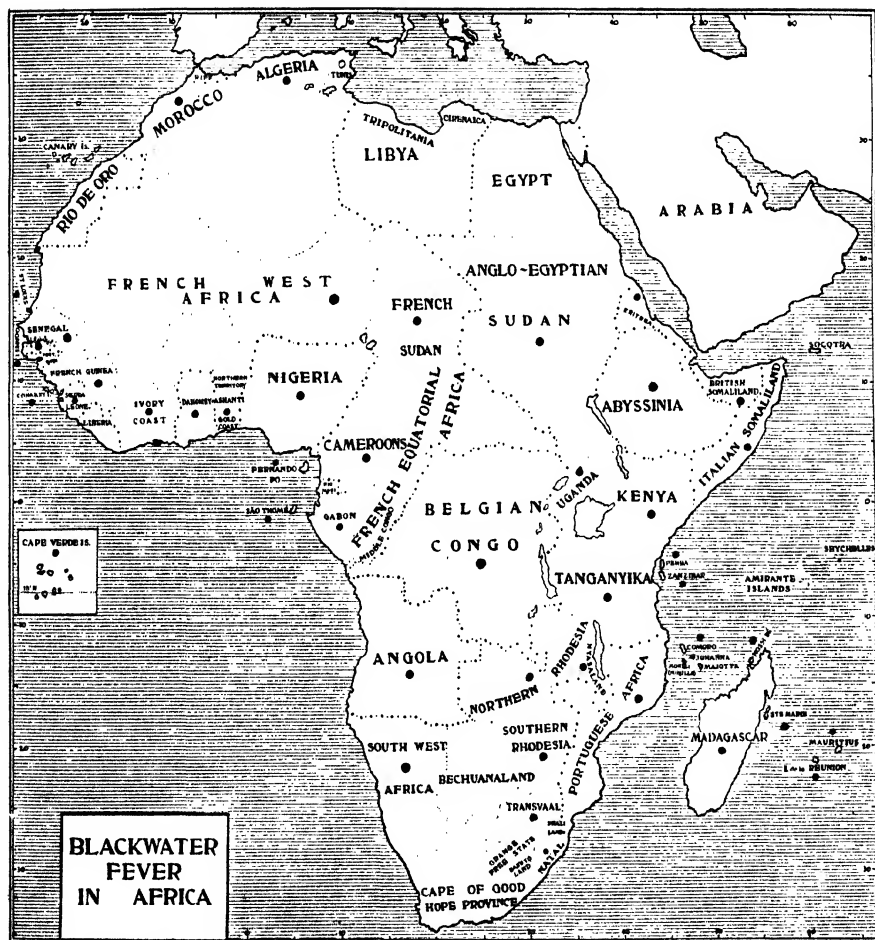
Europeans	Cases	Deaths	Native	Cases	Deaths	Year	Authority
							Zanzibar Protectorate
...	1	0	...	10	1	1915	(1916), p. 10.
...	2	5	1	1916	(1917), p. 11.
...	2	11	2	1917	(1918), pp. 3, 5, 10.
...	1	0	...	7	1	1918	(1920), p. 11.
...	0	...	196,733	7	...	1919	(1921), pp. 9, 31.
...	1920	
...	1921	
...	1922	
...	1923	
...	1924	
...	1925	
...	1926	
...	5 ¹	1927	(1928), p. 95.

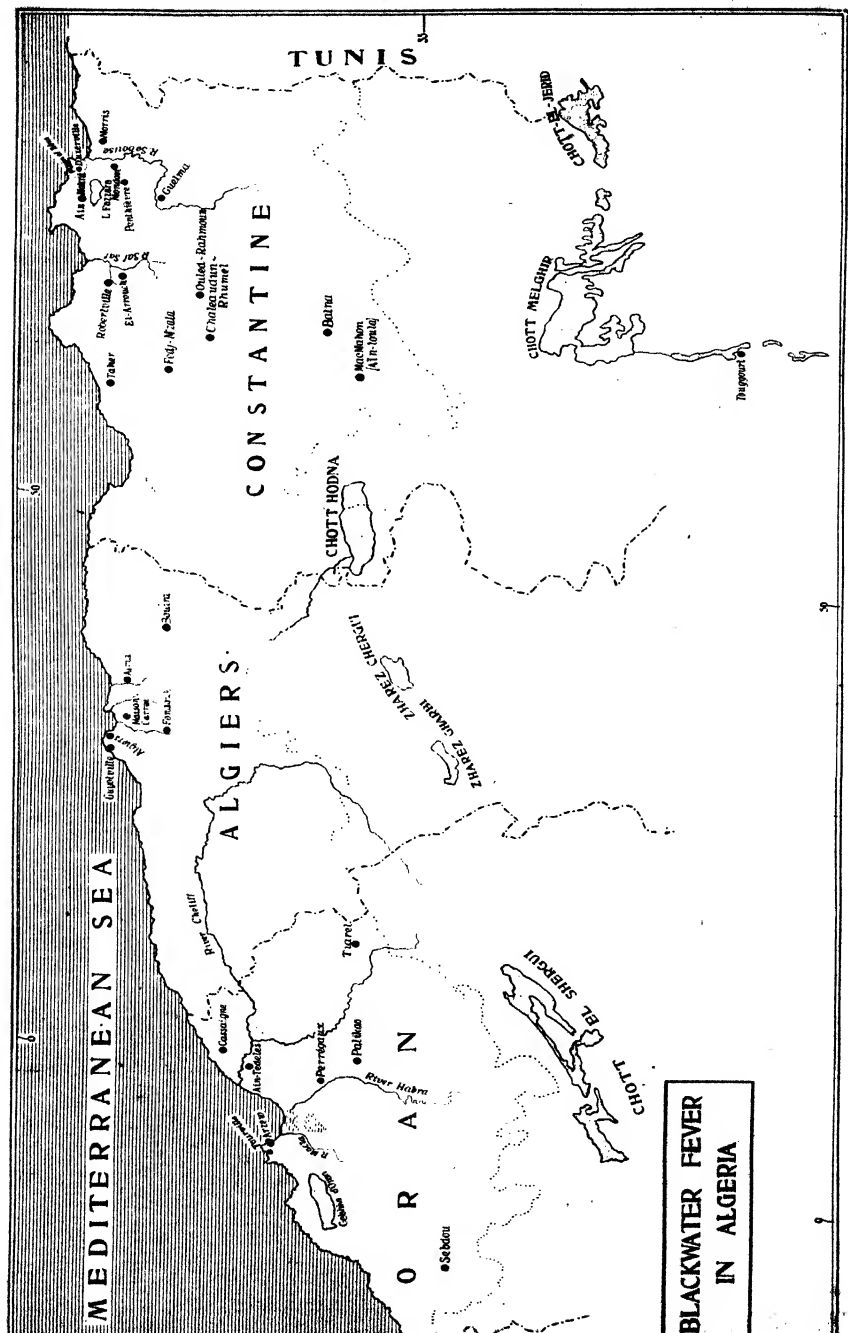
(1) Nationality not stated.

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SUSCEPTIBILITY AND RESISTANCE TO TRYPANOSOME INFECTIONS

V. THE RESISTANCE OF RATS TO INFECTION

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Host infection is conditioned on the one hand by the virus and on the other by the host. In the common type of infection the process may be said to consist of three phases. First there is the resistance to invasion—the virus being either prevented from entering the host or destroyed immediately on entry. As a consequence only a given percentage of those exposed to the virus become infected. If and when the virus gains entry into the body of the host there follows a period of lag or incubation, the length of which in any given host depends largely on the dosage and virulence of the virus. Finally there is the last and most important phase during which the host develops certain specific antibodies destructive to the virus, the final outcome depending to a large extent on the rate of evolution of these substances by the host in relation to the rate of multiplication and destruction of the virus.

In pathogenic protozoan infections such as malaria and trypanosomiasis, the host-parasite antagonism differs from that observed in bacterial infections. The first stages of the process are apparently similar; the last phase, however, is distinctly different. Even when spontaneous recovery occurs, the immunity is a transient one and specific antibodies are not readily demonstrable. As a rule, however, the host ultimately succumbs after a more or less prolonged struggle. The nature of this resistance to a protozoan infection and the various factors influencing it has been the subject of our studies.

At present there are two views. One, supported by Taliaferro (1922, 1926), maintains that the resistance on the part of certain animals is essentially the same as in bacterial infections, and that specific lytic antibodies are developed in the course of the infection. Our own results (1924, 1926) and those recently reported by Regendanz and Kikuth (1927), on Lewisi infections in splenectomized rats, indicate that the resistance is essentially non-specific in character and resides in the reticulo-endothelial system. Even the inhibitive substance discovered by Taliaferro, in Lewisi infections, is, according to Regendanz and Kikuth, not developed in splenectomized animals.

In this connection the variable resistance of different hosts to the same organism is of special interest. Different hosts react to same dosage per body weight in a different manner; the same dose of the same strain will produce a rapidly fatal disease in the mouse, a more prolonged infection in the rat, a relapsing type of disease in the guinea-pig, and a chronic infection in the rabbit. The end result is the same; the nature and duration of the process is different. Similarly the same dose of the same strain will cause a more rapid evolution of the disease in infant rats than in adults and in mal-nourished than in well-nourished animals. Variations in the dosage of the virus also produce different results. Larger or smaller numbers of organisms will produce a more or less rapidly fatal disease in mice (Doerr and Berger, 1922), and rats and a shorter or longer incubation period in guinea-pigs (Kligler and Rabinowitch, 1927).

It appears, therefore, that an understanding of the mechanism of resistance can be best obtained by a combination of the two methods. On the one hand, by observing the reactions of a given host under different conditions, and on the other, that of different hosts under the same conditions. In our previous contributions we followed the reaction in the guinea-pig and rabbit under various conditions with a view to observing the character of the resistance and, more particularly, the nature of the relapsing infection. It was shown that sensitising as well as immunising processes go on simultaneously, that the resistance can be modified artificially by injuring or blocking the reticulo-endothelial system, and that environmental and nutritive factors played an important rôle. (Kligler and Weitzman, 1926, Kligler and Geiger, 1928.)

For obvious reasons the influence of these latter factors can be more readily followed in the rat than in the guinea-pig. In the rat the character of the disease is simpler, the duration shorter, breeding and nutrition more easily controlled.

As a preliminary to these studies it was necessary to elucidate the nature and course of the infection in normal rats. In their studies on resistance to trypanosome infections the Taliaferros (1922) concluded that in the rat there is, as a rule, no evidence of any resistance being built up either against the rate of reproduction or toward the destruction of the parasites. If that were the case, one would expect to observe in the rat the same simple curve of geometric progression noted by Doerr and Berger (1922), in mice infected with *Trypanosome gambiense*. This is not, however, the case; neither the protocols presented by the Taliaferros, nor the preliminary counts made by us indicated that the progress of infection in the rat was of the same simple character as that observed in the mouse. On the contrary, it appeared that the rat manifested a definite resistance probably of the destructive type, which was not observed in the mouse.

Infections in mice and rats have one aspect in common. In both animals, once the trypanosomes establish themselves in the peripheral circulation, they increase progressively in number until the death of the animal. In the mouse the multiplication follows a geometric progression, the generation time remaining practically constant. In the rat, however, the progression is irregular and the generation time variable. These facts are illustrated by the protocols of Doerr and Berger for mice, and the Taliaferros', and our own observations for mice and rats. The problem to which we directed our attention was whether the variable progression in the rat was characteristic and, if so, what the nature of this resistance was.

Doerr and Berger (1922) assumed that the rate of multiplication in the mouse was a function of virulence. This is probably the case in the simple instance (the mouse) where the host is apparently absolutely incapable of offering any resistance to the invading parasite. In a more complex instance where the host does offer some kind of resistance, the rate of multiplication is a function of host resistance as well as virus virulence. If the virulence and dosage are kept constant, then a change in the rate of increase would

indicate that resistance is offered and that there is either an inhibitive or a destructive mechanism at work, if not both. At any rate, an idea of the extent if not the kind of resistance offered by the rat, may be obtained by a study of the rate of increase of various species of trypanosomes under comparable conditions.

Methods. The course of development of trypanosomes in infected rats was studied under conditions where other factors such as age, dosage, etc., were kept constant. Two strains of trypanosomes, *T. evansi* and *T. gambiense*, were used.

The infection was followed by counts made at frequent intervals. Early in the infection daily counts were made. As the infection advanced, counts were made at shorter time intervals.

The rats were always inoculated with a small number of organisms in order to obtain a true picture of the normal course of the infection. The injection of massive doses of virus is in itself sufficient to modify the course of the infection and lead to variable results. Blood taken from the heart was diluted first with citrate and then with saline, so that 0.5 or 1.0 c.c. contained the requisite number of organisms. Inoculations were always made intraperitoneally.

Two counting methods were used either singly or simultaneously. Direct counts were made in the blood counting chamber, by diluting the blood in saline to 1:100 or 1:200 as in red cell counts. If counts are made promptly while the trypanosomes are still alive and sluggishly motile, no difficulty is experienced in making the count. Usually we counted the number in 400 small squares, in order to reduce the error. Occasionally counts were made by comparing the number of trypanosomes with that of red cells in an ordinary blood smear stained with Giemsa stain. Because of the progressive anaemia in the course of the infection it is essential to make a red cell count prior to such counts. There was always close correspondence between the two methods and they could be used interchangeably. For counts at frequent short intervals the slide method is preferable because slides can be prepared, labelled and counted at leisure.

Results. The results were, in the main, so uniform that it will suffice to present a number of characteristic protocols.

I. Rats infected with *T. evansi*. (See also fig. 1.)

(a) Infected with trypanosomes from guinea-pigs.

EXPERIMENT 1. Rat, 98 gms. in weight, inoculated 5,000 *T. evansi* from guinea-pig, 19.2.28. The blood was positive on 29.2.28, after an incubation period of 10 days. Died 9.3.28. Duration of life 19 days.

TABLE I.

Date	Hour	Blood Count		Tryps. Count (per mm. ³)	Generation Time*
		R.B.C.	W.B.C.	Chamber	Hours
2.3.28	600	...
4.3.28	9.00 a.m.	5,780,000	7,500	2,400	...
5.3.28	8.30 a.m.	200,000	} { ∞ † 30 34
6.3.28	8.30 a.m.	290,000	
	2.30 p.m.	480,000	
7.3.28	9.30 a.m.	660,000	} { 17
8.3.28	9.00 a.m.	1,960,000	
	2.00 p.m.	2,070,000	} { ∞
9.3.28	9.00 a.m.	1,940,000	
	12.30 p.m.	1,790,000	...
9.3.28	p.m.
Average ...		5.3.28, a.m., 9.3.28, a.m.			32

* If we assume that the animal in question offers no resistance either of the destructive or inhibitive type, then the increase of the trypanosomes in the circulation ought to be in the nature of a geometric progression. In this case

$$An = A_0 \cdot r^{n-1} \text{ or } \frac{An}{A_0} = r^{n-1}$$

where An = number of trypanosomes at the end of a given time interval Tn .

A_0 = number of trypanosomes at the beginning.

r = the constant ratio, in this case 2, since the division of the trypanosomes is a simple one.

The only unknown in the equation is n , the number of intervals or divisions in time Tn .

The generation time equals the total time interval Tn , divided by the number of periods minus one, or $\frac{Tn}{n-1}$.

Example: On 6/3 8.30 a.m. the count was 480,000 and on 8/3 9.00 a.m. 1,960,000; the equation is, therefore, $\frac{1,960,000}{480,000} = 2^{n-1}$

$$4 = 2^{n-1} \text{ or } n-1 = 2.$$

$$\text{The generation time} = \frac{Tn}{n-1} = \frac{42}{2} = 21.$$

† The symbol ∞ is used to indicate that the apparent generation time is infinity. In reality a microscopic examination of a stained specimen reveals numerous dividing forms; what is presumably happening is that destruction and multiplication proceed at the same rate.

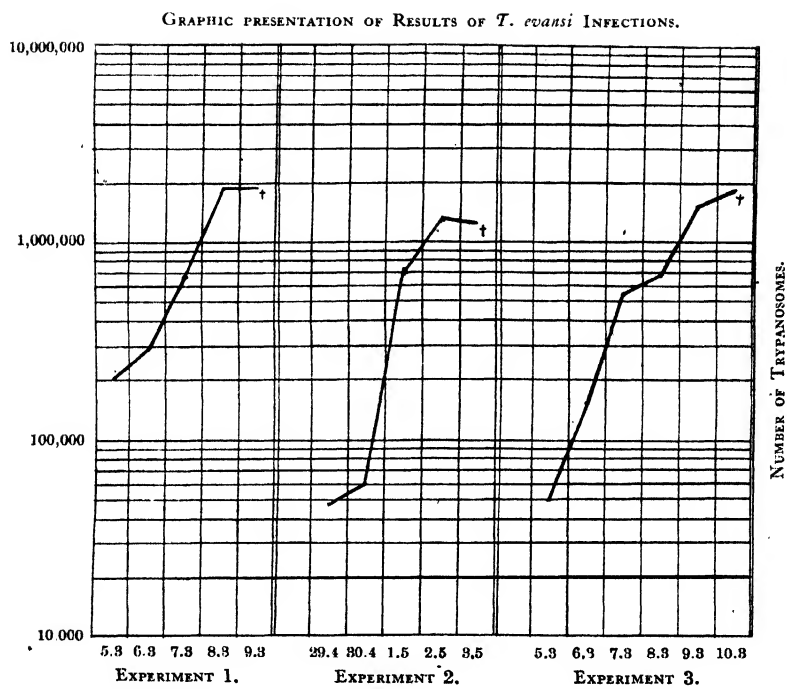


FIG 1.

EXPERIMENT 2. Rat, 184 gms., inoculated 20,000 tryps., (*T. evansi* from guinea-pig) on 17.4.28. Blood positive, 22.4.28. Duration of life 16½ days.

TABLE II.

Date	Hour	Blood Count	Tryps. Count	Generation Time
		R.B.C.	Chamber	Days
16.4.28	...	6,160,000
22.4.28	occ.	...
23.4.28	2,000	} ∞
24.4.28	9.45 a.m.	4,880,000	2,000	
25.4.28	9.15 a.m.	...	Neg.	...
	12.15 p.m.	...	2,000	...
26.4.28	10.00 a.m.	...	2,000	} 13½
27.4.28	1.10 p.m.	...	8,000	
29.4.28	10.00 a.m.	...	46,000	} 21
30.4.28	10.00 a.m.	5,020,000	60,000	
1.5.28	3.45 p.m.	4,590,000	698,000	} 18
	5.00 p.m.	
2.5.28	9.45 a.m.	5,050,000	1,180,000	} ∞
	12.00 noon	4,360,000	1,150,000	
	3.20 p.m.	4,340,000	1,230,000	
3.5.28	8.40 a.m.	3,360,000	1,166,000	} ∞
	11.55 a.m.	...	1,190,000	
	4.00 p.m.	3,000,000 Heart	1,024,000	
	4.55 p.m.	2,350,000	1,300,000	
3.5.28	4.55 p.m.

(b) Infected with trypanosomes from rat ; first passage from guinea pig.

EXPERIMENT 3. Rat, 88 gms., weight inoculated 5,000 tryps. from rat, first passage. Incubation 10 days. Duration of life 20 days.

TABLE III.

Date	Time	Tryp. Count	Generation Time	
			Hours	
29.2.28	...	+	...	
1.3.28	...	+	...	
2.3.28	...	+	...	
4.3.28	...	+	...	
5.3.28	2.00 p.m.	50,000	} 16
6.3.28	9.15 a.m.	150,000		
6.3.28	2.45 p.m.	290,000	} 21
7.3.28	9.00 a.m.	520,000		
8.3.28	9.00 a.m.	680,000	} 24
9.3.28	9.30 a.m.	1,540,000		
	1.00 p.m.	1,480,000	} ∞
10.3.28	9.00 a.m.	1,810,000		
	11.00 a.m.	1,680,000		...

+ = Trypanosomes present in stained drop but too small in number to count.

(c) Infected with trypanosomes from rat ; 3rd passage.

EXPERIMENT 4. Rat, 232 gms., inoculated 6,000 tryps. (*T. evansi*, 3rd rat passage) on 25.3.28. Blood positive 7.4.28. Incubation 13 days. Duration of life 18 days.

TABLE IV.

Date	Hour	Blood Count	Tryps. Count		Generation Time
		R.B.C.	Chamber	Smear	Hours
26.3.28	...	7,870,000
7.4.28	a.m.	7,480,000	28,000	}	∞
8.4.28	11.00 a.m.	5,950,000	34,000		
9.4.28	11.00 a.m.	...	138,000	}	12
	4.45 p.m.	...	318,000		
10.4.28	9.00 a.m.	...	410,000	}	16
	2.45 p.m.	3,840,000	556,000		
11.4.28	9.30 a.m.	3,910,000	950,000	}	24
	2.00 p.m.	...	930,000		
12.4.28	8.30 a.m.	4,100,000	1,254,000	1,391,600	...
	2.40 p.m.	...	1,200,000		...
	3.40 p.m.	2,680,000	...	1,188,000	...

(d) Infected with trypanosomes from rat ; 4th passage.

EXPERIMENT 5. Rat, 84 gms. in weight, inoculated 15,000 tryps. from rat, 4th passage. Incubation 10 days. Duration of life 22 days.

TABLE V.

Date	Time	Counts		Generation Time
		R.B.C.	Tryps.	Hours
26.4.28	...	5,740,000	Occasional	...
6.5.28
8.5.28	9.00 a.m.	5,480,000	Negative	...
9.5.28	9.00 a.m.	...	Negative	...
10.5.28	10.00 a.m.	5,000,000	4,000	} 18
11.5.28	10.00 a.m.	...	6,000	
13.5.28	10.00 a.m.	3,980,000	12,000	} 48
14.5.28	1.00 p.m.	...	28,000	
	6.00 p.m.	4,230,000	58,000	} 24
15.5.28	12.00 noon	4,100,000	76,000	
	6.00 p.m.	...	146,000	} 18
16.5.28	10.00 a.m.	4,200,000	284,000	
17.5.28	9.00 a.m.	4,230,000	588,000	} 24
	5.00 p.m.	3,980,000	780,000	
18.5.28	9.00 a.m.	3,700,000	1,150,000	} 24
	12.00 noon	3,440,000	1,200,000	
	1.15 p.m.	2,040,000	...	

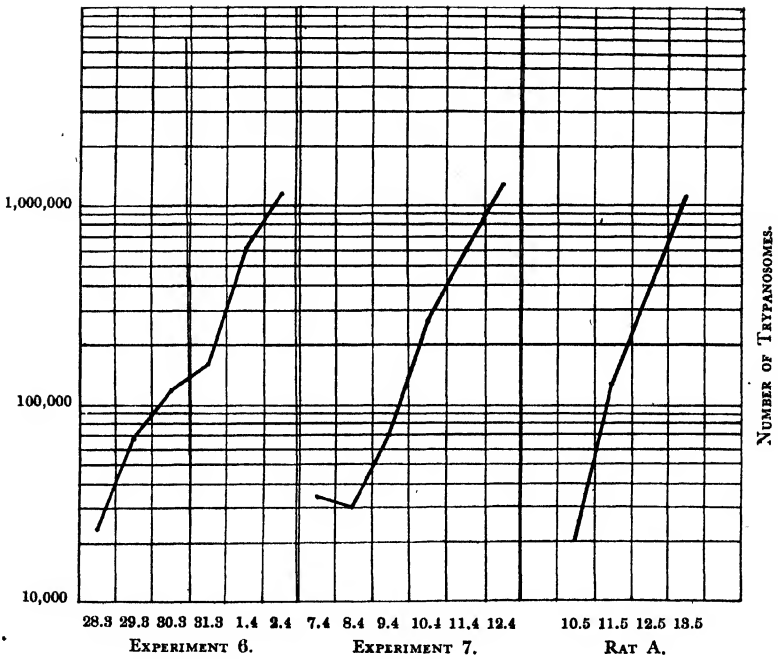
II. Rats infected with *T. gambiense*. (See also fig. 2.)

(a) Infected with *T. gambiense* from rat ; 2nd passage.

EXPERIMENT 6. Rat, 59 gms., inoculated 20,000 trypanosomes from rat, 2nd passage Incubation period 6 days. Duration of life 19 days.

TABLE VI.

Date	Hour	Count		Generation Time
		Red Cell	Tryps.	Hours
26.3.28	9.00 a.m.	7,960,000	2,000	9 a.m., 26-27 = 12
	5.00 p.m.	...	4,000	...
27.3.28	9.00 a.m.	...	8,000	...
	5.00 p.m.	...	16,000	9 a.m., 27-28 = 16
28.3.28	9.00 a.m.	...	22,000	...
	3.30 p.m.	...	42,000	9 a.m., 28-3 p.m., 29 = 15
29.3.28	9.00 a.m.	...	68,000	3 p.m., 28-3 p.m., 29 = 24
	12.00 noon	...	80,000	9 a.m., 29-30 = 24
	3.30 p.m.	4,360,000	86,000	...
30.3.28	9.00 a.m.	3,240,000	108,000	9 a.m., 30-31 = ?
	11.00 a.m.	...	130,000	...
	2.00 p.m.	...	96,000	9 a.m., 31-1 = 12
	3.30 p.m.	...	122,000	...
31.3.28	9.00 a.m.	...	156,000	...
	12.00 noon	...	156,000	9 a.m., 1-2 = 24
1.4.28	9.00 a.m.	...	602,000	...
	3.30 p.m.	...	712,000	...
2.4.28	8.30 a.m.	2,600,000	1,084,000	...
	9.15 a.m.	...	1,335,000	...

GRAPHIC PRESENTATION OF RESULTS OF *T. gambiense* INFECTIONS.

Note the contrast in the character of the first two curves as compared with the last which approximates that of the mouse.

FIG 2.

(b) Infected with *T. gambiense* from rat ; 3rd passage.

EXPERIMENT 7. Rat, 74 gms. in weight, inoculated 6,000 *T. gambiense* from rat, 3rd passage. Incubation 9 days. Duration of life 18 days.

TABLE VII.

Date	Hours	Count			Generation Time.
		R.B.C.	Chamber	Smear	Hours
7.4.28	a.m.	6,680,000	34,000	...	∞
8.4.28	p.m.	...	30,000	...	
9.4.28	9.00 a.m.	...	70,000	...	24
10.4.28	10.00 a.m.	...	256,000	...	12
10.4.28	3.00 p.m.	...	408,000	...	24
11.4.28	11.15 a.m.	3,360,000	600,000
12.4.28	11.15 a.m.	3,370,000	1,100,000	1,196,350	24
	2.15 p.m.	...	1,313,000
	4.00 p.m.	...	1,556,000
	5.30 p.m.	1,390,000 (Heart blood)	...

EXPERIMENT 8. Rat, 72 gms., inoculated 6,000 *T. gambiense* from rat, 3rd passage. Incubation 8 days. Duration of life 17 days.

TABLE VIII.

Date	Hour	Count		Generation Time
		R.B.C.	Tryps.	Hours
4.4.28	a.m.	...	20,000	12
6.4.28	a.m.	...	350,000	
7.4.28	9.00 a.m.	6,150,000	728,000	24
8.4.28	9.00 a.m.	5,680,000	660,000	∞
	1.00 p.m.	...	600,000	∞
	4.00 p.m.	...	700,000	
9.4.28	10.00 a.m.	...	600,000	24
	1.00 p.m.	...	458,000	
	3.30 p.m.	2,780,000	478,000	
10.4.28	9.00 a.m.	...	1,300,000	∞
	12.00 noon	...	778,000	
	1.00 p.m.	...	776,000	
	2.30 p.m.	2,800,000	684,000	
11.4.28	8.00 a.m.	...	1,620,000 (Heart blood)	

Discussion. It is apparent from the protocols and curves presented above that the infectious process in trypanosome infected rats is not a simple one. Before the organisms have definitely invaded the circulation, as well as after they have established themselves, there are fluctuations in the numbers of trypanosomes and the growth curve is quite different from that observed in mice. An analysis of the protocols presented by the Taliaferros shows that their results correspond with ours. Rats 709 and 729 show a definite relapsing type of infection, while Rats 703 and 705 manifest the same general irregularity in the generation time as do our rats. Another point of interest is that the same strain in mice not only shows a smooth growth curve, but a much lower average generation time than it does in rats. In the mouse infected by them with

T. rhodesiense, for example, the generation time fluctuates around 6 hours and the average is 6.5 hours, whereas in the rats presumably infected with the same strain, the generation time varies from 6 hours to 48 hours, and the average is 17 to 18 hours (Rats 703 and 705).

These irregularities can only be accounted for by assuming that the rat possesses some mechanism of resistance which does not exist in the mouse. This mechanism is probably similar in kind to that present in the guinea-pig, although different in degree. Experiments not yet completed indicate that the injection of olive oil has a similar depressing effect on the resistance of rats as it has on guinea-pigs. In the latter the Taliaferros postulate a destructive type of resistance because the coefficient of variation of the developing trypanosomes which is a measure of rate of growth is constant. That this is also the case in our rats is indicated by the fact that there is an active state of division as well as an actual rise and fall in the numbers of trypanosomes at all stages of the infection. If this assumption is accepted as valid, and the rate of multiplication is constant, then it follows that the variations in the generation time in infected rats at different stages of the process is due to a variation in the rate of destruction. This destructive process must go on in the rat throughout the entire period of infection, even in the early stages before the hypothetical antibodies have had time to develop, since variations in numbers are just as common in the early as in the late stages of the infection.

These experiments do not solve the problem as to the nature of the mechanism responsible for the destruction. Large amounts of serum taken from a rat at the height of the infection, and injected into another infected rat, produces no effect on the numbers of trypanosomes or on the course of the infection. At the same time, it is possible occasionally to obtain an infection in a treated rat which corresponds closely with that observed in mice. In one experiment a group of rats were infected with *T. gambiense*. The infection failed to develop, presumably because the dilution process left a smaller number of organisms in the inoculum than was expected. Three weeks later these rats were re-infected with 25,000 organisms of the same strain. All the rats of the series showed a lower incubation period (4.5 instead of 8 days) and shorter duration of illness (10½ instead of 18 days), and some of them showed growth curves

approximating that in mice (see fig. 2). The protocols of two of these rats is presented below. The others were less regular but of the same general character.

TABLE IX.

Number Weight	A 244 Count	Generation Time	B 66 Count	Generation Time
		Hours		Hours
Infected 12.4.28
Re-infected, 4.5.28
8.5.28, 8.20 a.m.
9.5.28,
10.5.28, a.m.	20,000	8
11.5.28, a.m.	128,000		44,000	12
12.5.28, a.m.	...	16	...	
13.5.28, 9.50 a.m.	1,032,000	...	708,000	6½
11.30 a.m.	1,320,000		...	
5.00 p.m.	...		1,401,000	
		Average 12		Average 11

Similar effects may be obtained by an injection of oil prior to an infection. In other words, rats treated so as to induce a depression of the resistance mechanism develop an infection which approximates that observed in the mouse. It would seem from these observations that we are dealing with a resistance mechanism which varies in degree in these different animals, being practically nil in the mouse, moderate in the rat and much more highly developed in the guinea-pig.

The cause of death in trypanosome infections is a question of considerable interest. Doerr and Berger called attention to the fact that in their mice death occurred when the number of trypanosomes reached a certain fairly constant concentration per cubic millimeter of blood. We observed a similar relation in our rats. The average number of trypanosomes per cubic millimeter at or shortly before exitus was 1,440,000 in 11 rats infected with *T. evansi*, and 1,420,000 in 14 rats infected with *T. gambiense*. It is not certain, however,

whether the number has a direct causal relation to the death and the shock which precedes it or whether the number is incidental to other concomitant factors which are responsible for death, the latter limiting the number of trypanosomes per unit volume. In this connection it is of interest that different species of trypanosomes apparently reach a different limiting concentration. From the protocols presented by the Taliaferros it appears that in infections with *T. rhodesiense* death ensues when the number per cubic millimeter is about 3,000,000. In *T. equiperdum* the number reaches 5,000,000 and in *T. equinum* 10,000,000. In our experiments with infant rats (11-12 gms.) death occurred when the number was only 750,000. It is noteworthy that in all cases the red cell count at time of death was greatly reduced, often to about 2,500,000, or about one-third of the original count. In experiments now in progress we have noted a constant increase in the lactic acid concentration of the blood parallel with the increase in the number of trypanosomes. These experiments will be reported in another communication.

Whatever the immediate cause of death may be, there is a direct connexion between it and the number of organisms in the blood stream. The difference in the final concentration of trypanosomes in infections with different species might be due to a difference in the rate of fermentation, so that the ultimate injury resulting in death is produced only by a larger number of organisms. If this proves to be the case, we have both a simple explanation of their pathogenicity, as well as a method for differentiating some of the species of trypanosomes.

SUMMARY

A study was made of the course of a trypanosome infection in rats and of the character of their resistance to such infections. The investigation was carried out by following the course of infection in rats by repeated counts of the number of trypanosomes in the circulation. An analysis of the data indicates that :—

1. The infection in the rat is of an order intermediate between that in the mouse and guinea-pig. The rat possesses a resistance which is probably the same in kind as, but different in degree from that of the guinea-pig.

2. Although the infection is a progressive one there is a constant destruction of trypanosomes throughout the course of infection.

3. Depression of the resistance may result in an infection which approximates more closely that of the mouse. This phase of the question is now under investigation.

4. At time of death the concentration of trypanosomes in the circulation is constant. For the species studied, the average numbers were 1,440,000 for *T. evansi*, and 1,420,000 for *T. gambiense*. The red cell count is also greatly reduced, but varies a great deal more than the trypanosome count.

The direct causal relation of these factors to death of the animals is not certain. It appears, however, that death is due to injury produced by the metabolic products of the trypanosomes (lactic acid) and that the organisms must reach a certain concentration, which differs with different species, before they can affect the changes or intoxication which lead to death. Disturbance in the oxidation mechanism is probably a critical element in the process.

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THE EARLY LIFE HISTORY OF *CREPIDOBOTHRUM TESTUDO* (MAGATH 1924)*

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PLATE III

In 1924 I described a cestode as *Ophiotaenia testudo*, from the soft-shelled turtle (*Amyda spinifera*). Nybelin's (1917) paper on Australian cestodes, which were collected during Dr. Mjöberg's Swedish Scientific Expedition to Australia, was not available to me and I did not know that he considered the genera *Ophiotaenia*, *Ophiodotaenia* and probably *Solenotaenia* as synonymous with *Crepidobothrium*. Although it is not necessary in this paper to consider his arguments for such synonymy in regard to *Ophiodotaenia* and *Solenotaenia*, it is desirable to point out that he considered La Rue's genus *Ophiotaenia* not to possess sufficient distinguishing characters to separate it from the earlier named genus.

La Rue, in discussing the validity of *Ophiotaenia*, stated that 'while in the structure of the proglottids and in the arrangement of the genital organs this species (*C. gerrardii*) agrees almost perfectly with the *Ophiotaenia*, there remain two characters which are deemed of sufficient value to warrant a separation of the snake Proteocephalids into two genera. These characters are the structures of the suckers and the length of the neck.'

Nybelin found in certain individuals of *C. mjobergi* that some of the suckers were re-entrant and some were not, thereby negating one of the two characters pointed out by La Rue. The other character, the length of the neck, does not appear to be of sufficient importance to justify the separation of the genera. The most that could be said concerning the length of the neck would be that it might play a part in the separation of species but when one realises the great variation in the length of various organs and parts in an

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individual species, it is evident that the length of so elastic a structure as the neck is not of generic importance. Furthermore, in the generic characterisation of *Ophiotaenia*, La Rue does not mention the neck. There being no other distinguishing characteristics in the genus as opposed to the genus *Crepidobothrium* it appears that Nybelin's contention is valid.

Meggitt (1927), in discussing the validity of certain genera belonging to this group of worms, agrees with Nybelin and with him places the three genera in question as synonymous with *Crepidobothrium*. He accepts the genus *Ichthyotaenia* Lönnberg (1894), in preference to *Proteocephalus* Weinland (1858). The only other three genera in the family which he accepts are *Corallobothrium* Fritsch (1886), *Gangesia* Woodland (1925), and *Crepidobothrium* Monticelli (1899); the latter, in addition to the three genera already mentioned should according to Meggitt contain *Acanthotaenia shipleyi* Linstow (1903). As synonyms of *Ichthyotaenia*, he gives *Acanthotaenia* Linstow (1903), *Batrachotaenia* Rudin (1917), and *Choanoscolex* La Rue (1909).

It is unfortunate that Meggitt's (1927) article has suffered from what is evidently poor proof-reading for it often leaves one somewhat in doubt as to the author's meaning. Besides the fact that he has given as many as three different dates for the naming of a genus and two different spellings for the name of a species and has stated that the only *Corallobothrium* species is *lobosum* Riegenbach (1895), a similar error being made by Woodland (1925), there are certain other more serious errors in the text. He states that in *C. testudo* (Magath, 1924) there are from fifteen to twenty uterine pouches, whereas the original description states that the number of pouches is from fifteen to twenty-five. After arguing, on page 81, that the species *testudo* should be placed in the genus *Crepidobothrium*, he places it in the genus *Ichthyotaenia* on a subsequent page. This is evidently a curious error because in the key of species of the latter genus he lists *I. magna* (Magath, 1924); then in the table of species he lists the species as *I. testudo* (Magath, 1924). Evidently it should be the species of Hannum (1925). On page 77 he states that *C. gerrardii* (mis-spelled *gerrardi*) and *C. paraguayensis* appear to be identical in spite of the fact that *C. gerrardii* and *C. perspicua* are the only two members of the group given in his table 'D' that possess an apical organ and the genital pore in *C. paraguayensis* is anterior

and in *C. gerrardii* is central. In short, there is as much difference as given in his own table between these two species as any other two. He further says that the species '*magna*, '*mönnigi*, '*schultzei* [mis-spelled *schultzi*], '*testudo* and '*hylae* are stated to have the testes in two fields, but with the vagina anterior or posterior: no statement is made whether the vagina is invariably constant in position; only in '*filaroides* is this the case.' In the description of *C. testudo*, I stated on page 46, 'The vagina always lies anterior to the cirrus pouch.' Finally, one should point out the fact that his descriptions of the new species do not differ in general from those of previous authors.

Sandground (1928) described two new species of cestodes and re-described a third, all of which he placed in the genus *Ophiotaenia*. He did not discuss the validity of the name except to show that he was cognizant of the work by Nybelin, Woodland and Meggitt, but he did point out that Fuhrmann (1924) still retains La Rue's genus. However, on examination of Fuhrmann's paper one finds that while it contains a description of a species there is no discussion of the validity of the genus *Ophiotaenia*. Fuhrmann did not refer to Nybelin's work which might be taken to indicate that he had not examined it.

While it does not seem wise at this time to accept Woodland's (1925) sweeping revision of the Proteocephalids, although there is argument in favour of it, it seems to me clear that *Ophiotaenia* is synonymous with *Crepidobothrium*.

Being impressed by the fact that the only identifiable food in the stomach of more than one hundred soft-shelled turtles examined were crayfish, and that no crayfish had been seen in the more than two hundred hard-shelled turtles examined, together with the fact that *C. testudo* was never encountered in hard-shelled turtles and was present in soft-shelled turtles, I suggested that the life-history of the worm might have something to do with crayfish. Further work on this line had demonstrated that the crayfish probably does not enter into the life-history of the species.

Profiting by the excellent work done on the early life-history of certain cestodes by Essex (1927, 1928), I have investigated the life-history of *C. testudo* and am now able to describe the first stages. For the history of the subject and general procedure the reader is referred to the papers by Essex.

Eggs may be obtained in large numbers by placing pieces of the

adult worm in tap water, and masses of eggs may be seen to be extruded all along the worm. The description of the eggs which was given earlier (1924) was made from preserved material, hence the following description made from living material is more accurate. The size of the outer membrane varies a great deal, but in mature eggs (Plate III, fig. *a*) it ranges from 60μ to 100μ in diameter. This membrane is thin and transparent, containing thin mucoid fluid which contains the oncosphere surrounded by another covering. This covering consists of a membrane beneath which is a layer of granular material in which one sees globules of some more refractive material. This covering is about 10μ thick and inside it is the oncosphere. So far as I am able to see, there is no third membrane between the second membrane and the oncosphere. In this I agree with Essex (1928) and further consider the second covering homologous to the shell of such eggs as *T. saginata*. There is certainly no delimiting inner membrane to the granular second covering for if it is ruptured the contents flow out, leaving only the thin outer membrane of the second covering, and although I tried repeatedly, it was never possible to demonstrate a third covering.

The six-hooked oncosphere has an average diameter of 20μ and, with the second covering, is 40μ in diameter.

The mature embryo is motile within the second membrane but seems unable to free itself from the shell. The six hooklets invariably have the blade of the hooklet toward the centre of the oncosphere and the hooklets are in pairs. They are about 5μ long and the hook itself appears to be little more than a slight projection from, or knob on, the shank.

Following the usual procedure, a large quantity of eggs was placed in an aquarium with various samples of plankton. This particular material contained at least three unidentified species of Cladosera, one species of *Diaptomus* and *Cyclops leuckarti* and *Cyclops bicuspidatus*. During the course of the experiments two hundred Cladoserae and twenty Diaptomi were examined, but none was found to contain the larval forms of *C. testudo*. The two species of Cyclops ate the eggs of the tapeworm readily in the manner described by Essex (1928). However, no development took place in *C. bicuspidatus*. In one experiment, after feeding *C. bicuspidatus* with eggs, seven were examined 72 hours later, six after 96 hours, twelve after 110 hours, and five after 132 hours, with negative results.

On the other hand, every *C. leuckarti* examined (eighty-two in all) after it had been in contact with these eggs, contained the developing oncosphere in numbers from one to twelve. In view of this, together with the fact that the development of the oncosphere was progressive with time, supports the conclusion that *C. leuckarti* is a natural first intermediate host for *C. testudo*.

As soon as the oncosphere reaches the body cavity of the Cyclops, which takes place in at least six hours, it has become quite active, extending and contracting, but for the most part retaining a spherical shape. The hooklets, now fully formed and about 10μ long (Plate III, fig. *d*), can be thrust out from the limiting membrane and now it is observed that the larvae seem to move with the hooklets at the anterior end, but the hook part of the hooklet is now near the periphery of the animal, opposite its position when the oncosphere was enclosed in its shell. This fact I have never seen referred to and since the hooklets have never been seen to turn over, it must be concluded that they immediately begin to pass backward through the body of the oncosphere. Essex (1928) illustrated the same thing in his drawing, but did not comment on it. Hunter (1928) has represented the hooklets in the same relative position in the egg (opposite to the way I found them) as in the early larva.

The motion of the hooklets is interesting. As the larva contracts they appear with the two lateral pairs horizontal and in the same straight line, with the central pair at right-angles. When the larva elongates, the three pairs become parallel. This alternating contraction and extension is often quite rapid.

Variation in size in the growing larvae is great and depends somewhat on the number the Cyclops ingests, hence the figures given will be for typical oncospheres at different stages. They were studied alive in 0.5 per cent. solution of sodium chloride because plasmolysis took place in water. Plate III, fig. *b*, illustrates an oncosphere which had been fed 47 hours earlier and it was 40μ in diameter. After another forty-eight hours a Cyclops was examined which harboured ten oncospheres, the largest being 60μ in diameter (Plate III, fig. *c*), and still spherical. After that, the larvae elongate and the hooklets remain posterior. After another twenty-four hours the largest larvae appear as in Plate III, fig. *c*, and have an average length of from 0.160 mm. and 0.090 mm. wide. They are capable of a great deal of extension and begin to assume more of a ribbon shape.

Hooklets are often left in the body of larvae and after another forty-eight hours have elapsed the end away from the hooklets is definitely established as the head end. However, the larvae have practically reversed their polarity after the ninety-six hour stage. The form is capable of constricting at irregular intervals so that the hooklets eventually are contained in a small rounded ball at the posterior end and the head end is often pushed out in a knob-like form. This does not become permanent until the next day when the so-called end organ appears as an urn-like structure with an anterior indentation. Eight days after the Cyclops has been fed, the end organ is fully formed and is 50μ in diameter. It can be protruded and retracted. At this time the cercomer is formed, containing the hooklets, and is 25μ long. On the seventh day calcareous bodies appear and are arranged in two lateral rows (Plate III, fig. *f*), about eight in each row. The larvae are very active, moving constantly in the body of the Cyclops. They constrict themselves at different places and elongate, changing into grotesque shapes.

The next stage in their development takes place during the next twenty-four to forty-eight hours, when the four suckers make their appearance (Plate III, fig. *g*). They are especially noticeable when the larvae are elongated and appear as crescentic thickenings 38μ long. Although the embryos assume various shapes there is a definite tendency to constriction in a region which may be termed the neck. This accentuates the head and the body assumes an elongated appearance. There is further development of the end organ and suckers during the next few days, and the calcareous bodies become irregularly scattered throughout the body, but there is none in the head.

When the larva is twelve to thirteen days old its motility becomes less and it begins to contract more and more until finally the head invaginates into the body and the larva assumes the shape of a pear (Plate III, fig. *h*). The head often rotates (Plate III, fig. *i*) so that as one looks at the larva from the side, the end of the head presents itself. In this position the end organ and suckers are clearly defined. The larvae average 0.180 mm. long and 0.120 mm. broad at the greatest diameter. The end organ is 50μ in diameter and the suckers 38μ in diameter. After fourteen days the larvae cease to undergo further development except that the cercomer drops off.

The larvae are incapable of locomotion, but a churning movement of the protoplasm and the head is seen.

The part of the life-history between this phase and the appearance of the worm in the intestine of the turtle must for the present remain a matter of speculation, as must the nature and disappearance of the end organ. I have seen larval cestodes in the livers of fishes, which have heads suggesting the genus *Crepidobothrium* but possessing end organs. It may be that the infested Cyclops, when eaten by fishes, are freed of their parasites and that the worms find their way to the liver, there to encyst and be eaten in turn by turtles. I have been unable to ascertain whether soft-shelled turtles eat fish and I have never seen one in their stomachs, but they are swift enough to catch them and they may even obtain the plerocercoids from dead fish, or, of course, they may obtain the worm directly from the Cyclops. The large numbers of tapeworms seen in turtles and the failure to observe very small worms in the intestine rather argues against the last method of infestation. This subject will be studied later.

SUMMARY

The author agrees with Nybelin that the genus *Ophiotaenia* is synonymous with *Crepidobothrium*.

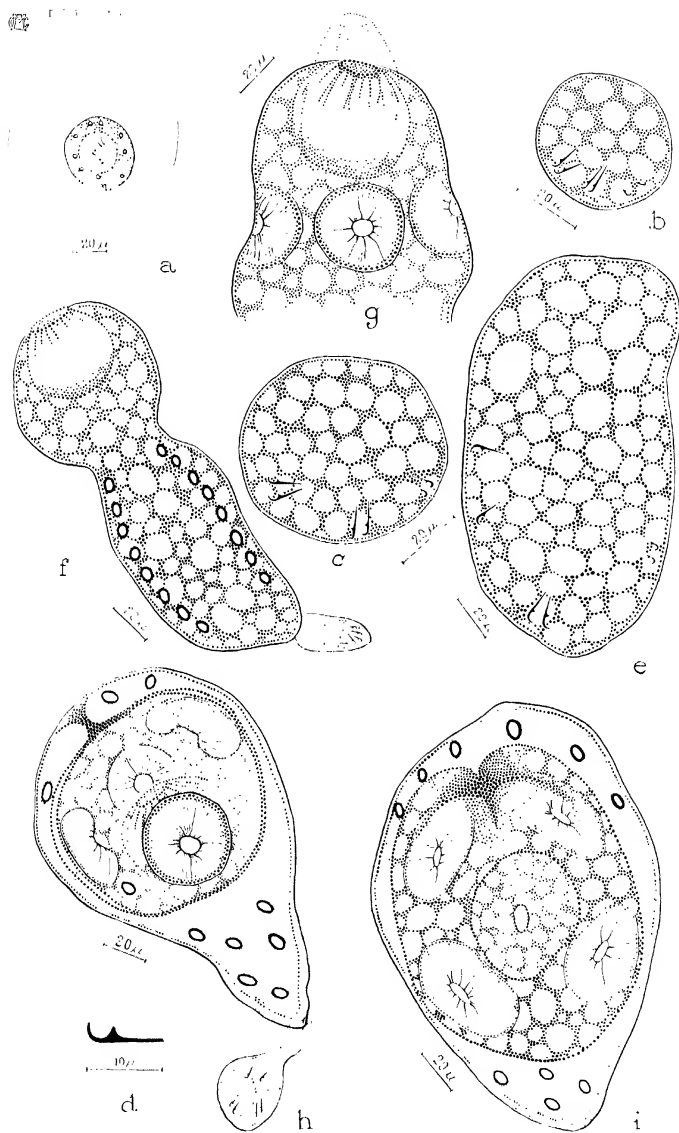
The early life-history of *C. testudo* (Magath, 1924) is described. It takes place in *Cyclops leuckarti* in fourteen days.

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EXPLANATION OF PLATE III

- Fig. *a*. Mature ovum of *C. testudo*.
- Fig. *b*. Embryo from *C. leuckarti*.
- Fig. *c*. Embryo from *C. leuckarti*.
- Fig. *d*. Hooklet from embryo.
- Fig. *e*. Embryo from *C. leuckarti*.
- Fig. *f*. Embryo from *C. leuckarti* showing cercomer and end organ.
- Fig. *g*. Head of embryo from *C. leuckarti* showing end organ and suckers.
- Fig. *h*. Embryo from *C. leuckarti* after head has invaginated.
- Fig. *i*. Embryo from *C. leuckarti* after cercomer has dropped off.



MISCELLANEA

STRONGYLOIDOSIS OF THE WOOLLY MONKEY (*LAGOTHRIX HUMBERTI*)

(Received for publication, 13 February, 1929)

In November, 1928, one of us (A. W. N. P.) received the stomach and intestines of a Central American monkey, which had died after showing marked vomiting, diarrhoea, and great loss of flesh. After some correspondence we think it was shown that the species in question was Humboldt's Nigger Monkey, or Woolly Monkey (*Lagothrix humboldti*). This species is often kept as a pet in this country.

On the outside of the small bowel, a male and female *Dipetalonema gracile* were found.

These long, and slender filariid nematodes live within serous cavities, and rarely produce disease.

The faeces contained numerous *Strongyloides* eggs, and scrapings from the mucous membrane showed large numbers of parasitic females of this genus. The following average measurements were obtained from five specimens: length, 4.86 mm.; length of oesophagus, .86 mm.; vulva to tip of tail, 1.94 mm.; anus to tip of tail, 81 μ . The vulva and anus were salient, and the striations were well marked. It is, therefore, suggested that the species is *S. papillosus* in the widest sense, and in accordance with the views expressed by Chandler (1925).

A damaged ancylostome was also found, which proved to be *Ancylostoma duodenale*.

Although there is some dispute as to the pathogenic effects of the different species of *Strongyloides*, it would appear from the history, the clinical account of the case, and the details of the post-mortem examination furnished by the sender, that the monkey's illness and death were due to intestinal toxæmia resulting from *Strongyloides* infestation. A point of additional interest is the presence of the human ancylostome in an animal which enjoyed the company of man.

A. W. N. PILLERS.

T. SOUTHWELL.

REFERENCE

CHANDLER, A. C. (1925.) The species of *Strongyloides* (Nematoda). *Parasitology*, **17**, 426.

A NOTE ON A NYMPHAL LINGUATULID— *LEIPERIA CINCINALIS* SAMBON—FROM THE MUSCULATURE OF THE FISH *TILAPIA* *NILOTICA*

(Received for publication, 13 February, 1929)

In July, 1928, Mr. S. C. J. Bennett, M.R.C.V.S., of Khartoum, forwarded to one of us (A. W. N. P.), a pentastome which he had collected from a cyst in the flesh of a river fish known on the Upper Nile as bulti (*Tilapia nilotica*). The preservative was apparently alcohol of unknown strength.

The specimen was a nymph measuring about 3.5 cms. in length, by 2 mm. in diameter at the thicker anterior end, and 1 mm. at the narrower posterior extremity. It was cylindrical, and slightly flattened in front. The colour was reddish-yellow. Throughout its length the cuticle was annulated, there being about 110 equidistant grooves.

The anterior extremity was rounded, and slightly bent towards the ventral aspect. Just behind the anterior border there were four openings, the two foremost of which were close together, whilst the posterior pair were more widely separated. From each of these openings there protruded a yellowish coloured, smooth, common trunk, which branched into two curved and sharply-pointed hooks. The anterior branch in each case was smaller and less curved than the hinder one. The smaller (anterior) measured about 210μ in length, and the larger about 250μ .

According to Diesing, the mouth is between the origins of the two hinder double hooks.

No further details of the structure could be made out. Taking into consideration the host, and the locality from which it was collected, it appears that the nymph can be placed in the 'group' *Pentastomum gracilis*. This name, according to Sambon (1922), has been applied to a number of immature linguatulids belonging to the genera *Sebekia* and *Leiperia*, of the Sebekini division of the sub-family POROCEPHALINAE.

The hooks in the present specimen were very similar to those figured by Sambon for the adult *Leiperia cincinalis* from the Nile crocodile, and it is therefore considered that the nymph belongs to this species.

T. SOUTHWELL.

A. W. N. PILLERS.

REFERENCE

SAMBON, L. W. (1922.) A synopsis of the family Linguatulidae. *Jl. Trop. Med. & Hyg.*, **25**, 188.



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STUDIES ON THE TREMATODE—FAMILY *HETEROPHYIDAE*

BY

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Jerusalem)

(Received for publication 20 May, 1928)

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I. INTRODUCTION

The family HETEROPHYIDAE Odhner, and its individual members, have repeatedly attracted the notice of many writers. Zoologists have called attention to their peculiar anatomical structure, while medical men have studied these worms because some of them are parasites of man and domestic animals.

In spite of a rather large literature, including several monographs devoted to *Heterophyidae*, there still remain many obscure points in their anatomical structure, life-history and classification. In the present paper it is hoped to clear up the existing discrepancies on the basis of investigations on the Palestinian representatives of the *Heterophyidae*.

Palestine proved to be rich in *Heterophyidae*, for fourteen members of this family have so far been found in the country. Nine of them have already been described by different authors, the remaining five proved to be new to science.

It is of interest to point out that while six out of the nine known species have been found in neighbouring countries (Egypt and Italy), the other three, namely *Monorchitrema taihwei* Nishigori, *Monorchitrema taihokui* Nishigori, and *Parascocotyle longa* (Ransom) are described from the other hemisphere—the first two from Formosa, the last from Alaska.

Material on which the present paper is based was collected from animals caught in Jerusalem, Tel Aviv, and the vicinity of Tiberias; in addition animals, mainly dogs and cats, were infected experimentally and the life-history in the final host studied.

Thus the intermediate hosts of the majority of these trematodes were detected. Investigations on the earlier stages of *Heterophyidae* have also been inaugurated but they require more time to be completed and the results will be published later.

The writer had the opportunity of using some cotype specimens generously sent to him by Dr. W. Arndt from the Berlin Museum, by Professor J. Ciurea from the University of Bucharest, by Dr. L. Szidat from the Königsberg Museum, and by Professor M. C. Hall and Dr. A. Hassall from the Bureau of Animal Industry, Washington.

The author takes this opportunity to express his gratitude to all these gentlemen for their kindness.

II. GENERAL REMARKS ON THE LIFE CYCLE OF THE *HETEROPHYIDAE*

The life-history of several species of *Heterophyidae* have already been studied by different authors. It was found that cercariae develop in snails. From the snail the cercaria reaches a fish, encysts in the organs of the latter and becomes a metacercaria. The latter after being swallowed by the final host gives rise to the adult worm, which parasitises the intestine.

My experiments with Palestinian *Heterophyidae* confirmed this general course of development which appears to be constant and typical. I also convinced myself that the majority of species are not specific in their choice of hosts. The metacercariae of *Heterophyidae* may encyst in various fishes belonging not only to different genera but also to different families. Similarly many species of *Heterophyidae* are not particular in their choice of a final host as they may develop partially or completely in both mammals and birds. A certain degree of specificity regarding the final host has, however, been observed. Faust and Nishigori have already pointed out that the species of the genus *Monorchitrema* may develop in some final hosts only up to a certain degree, and are then passed out. In my experiments, the metacercariae escaped from their cysts in all laboratory animals, but while in some the full development occurred and the worms reached maturity, in others the Trematodes lived only a short time and passed out before reaching maturity.

Further, even among the final hosts in which the worms can reach maturity, some are more suitable than others. In the more suitable hosts the parasites grow to a large size and produce many eggs, in the others the development is feeble and the worms are small. This phenomenon led the earlier investigators to misinterpret the specific characters of *Heterophyidae* and to consider one species as several according to the number of hosts in which it occurred. In my experiments the *Heterophyidae* developed in dogs better than in cats, in cats better than in rabbits. The same species found in mammals are in most instances better developed than in birds.

As the development of Palestinian *Heterophyidae* is similar in all species, a detailed description is given only for *Heterophyes heterophyes* (Siebold), which may serve as a model. The cited

numbers of secondary hosts of particular species of *Heterophyidae* should not be considered as complete, as only few out of very numerous species of fishes occurring in Palestine were used for the experiments.

III. GENERAL REMARKS ON THE CLASSIFICATION OF THE *HETEROPHYIDAE*

HISTORY OF THE FAMILY

The name *Heterophyidae* was given by Odhner (1914) to replace the former incorrect names *Cotylogonimidae* and *Coenogonimidae*. Odhner included in this family the genera which have been attributed to it by previous authors and several others the systematic position of which had been uncertain. The first attempt to gather all known species into a closed group was made by Ransom (1920), who gave a new modified diagnosis but divided the family only into genera, though the outlines of three sub-families had been already defined by previous authors. Ciurea was the first who defined the division of the *Heterophyidae* into sub-families. He differentiated the following five sub-families: *Heterophyinae*, *Metagoniminae*, *Centrocestinae*, *Apophallinae*, and *Cryptocotylinae*, basing himself on the structure of the terminal portion of the genital ducts. Later Faust and Nishigori (1924) added a sixth sub-family—*Monorchitreminae*. Meanwhile, Nicoll (1923) included the sub-families *Microphallinae* and *Gymnophallinae* as well as some genera which up to this date have not been attributed to it. Poche (1926) added several more families and genera to the *Heterophyidae*. According to the latter author, whose contribution summarises all the present knowledge of *Heterophyidae*, this family should consist of very differently organized forms which deviate considerably from the type genus.

STRUCTURE OF THE GENITAL PORE IN *HETEROPHYIDAE*

A comparative study of the anatomical structure of various members of this family led me to the conclusion that the peculiarities and extent of this family require to be cleared up, the significance of some of its typical characters must be defined and some genera removed from it. The most typical character of *Heterophyidae* is

the structure of the terminal portion of the genital ducts and the genital aperture or so-called 'genital sucker.' The term 'genital sucker' is attributed by authors to rather dissimilar structures which should be differentiated. In the genus *Heterophyes* this organ is a large protrusible body which may be retracted into a sac-like hollow which appears to be the homologue of the genital sinus of other Trematodes. This complicated structure, unlike the ventral sucker, possesses no adhesive functions and therefore the term 'genital sucker' seems to me to be a misnomer. I propose to name the protrusible body 'gonotyl,' while the cavity into which it may be retracted should be called 'genital sac,' as proposed by Yokogawa, 1913. (Fig. 7.)

The genus *Heterophyes* is the only one of the family *Heterophyidae* which has a ventral sucker independent of the genital sac. In other genera the ventral sucker is more or less reduced or modified and included in the genital sac, together with the gonotyl, which is reduced proportionally to the development of the ventral sucker ('Sphäroider Körper' of Jägerskjöld). All these structures form together a peculiar organ for which the term 'ventro-genital sac' is proposed. In some genera in which the well-developed gonotyl occupies the whole ventro-genital sac the ventral sucker may disappear completely (*Stictodora*, *Monorchitrema*, etc.). In other genera in which the ventral sucker is well developed the gonotyl is reduced to a comparatively small muscular tubercle (*Pygidiopsis*, etc.) or to two tubercles (*Apophallus*, etc.).*

These tubercles have been observed by the earlier authors who called them 'lenticular shaped bodies,' but their position has been misinterpreted. They are situated not on the ventral surface of the body, but inside the ventro-genital sac from which they may be occasionally protruded.

While in the members of the genus *Heterophyes* the genital aperture is situated on the top of the gonotyl, in other genera it lies at the base of this organ. In the majority of species the gonotyl bears chitinous spines, plates, bars, etc., on its surface.

* The *Heterophyidae* are not the only Trematodes with such a complicated genital sinus. Similar structures are also present in other groups of Trematodes, as for instance *Microphallinae*, *Hemiuridae*, *Axygiidae*, etc.

DIAGNOSIS OF THE FAMILY *HETEROPHYIDAE* Odhner.

On the basis of material worked out in this paper together with previous literature the family *Heterophyidae* may be defined as follows :

Small and very small forms. Pseudodermis covered with scale-like spines. The body is usually divided into two parts, one anterior flattened, free from genitalia and more motile than the posterior part which is oval or round in cross-section and contains the genital apparatus. The oral sucker may be provided with all or a part of the following structures : a contractile dorsal lip-like appendage, a posterior funnel-shaped appendage and rows of circumoral spines.

Praepharynx and oesophagus vary in different genera and species. Pharynx always present. Intestinal caeca simple, of varying length. Ventral sucker, except in the genus *Heterophyes*, reduced and included in the modified genital sinus (' ventro-genital sac ') or even absent.

The reproductive organs, except the vitellaria in some genera, are grouped in the posterior part of the body behind the level of the genital aperture which is generally situated near the middle of the body. Testes, two or one, globular or lobed : their situation varies in different genera. The cirrus pouch is absent. The seminal receptacle is voluminous and may be divided into several parts by constrictions. The terminal portion of the seminal vesicle may form a separate vesicle-shaped organ which is usually provided with chitinised walls ; the term ' expulsor ' is proposed for this structure (in *Heterophyes*, *Tocotrema*, *Diorchitrema*, etc.). Ovary globular or slightly lobed and, except in *Adleria*, is situated in front of the testes. Mehlis' gland present. Seminal receptacle well developed. Laurer's canal usually reduced. The vitellaria are usually situated near the lateral or dorsal surface of the body and the degree of their development varies in different species. The uterus in most cases does not proceed anteriorly to the genital aperture. The latter, except in *Heterophyes*, opens on the inner wall of the ventro-genital sac, which is situated on the middle line or moved towards the lateral border of the body. Near the genital aperture a more or less developed gonotyl is often present. Eggs usually numerous with

thick shell 18 to 37 μ long. Excretory vesicle usually Y-shaped; the length of the stem varies in different genera and it is either straight, S-shaped or divided into branches which may re-unite (as in *Scaphanocephalus*); the branches may be long, short or entirely absent (as in *Galactosomum*).

Adults parasitise the intestines of mammals, birds, and rarely fish (*Haplorchis*). Metacercariae encysted in fish. Cercariae, as far as is known, develop in operculated molluscs.

Type genus:—*Heterophyes* Cobbold, 1866.

CONTENTS OF THE FAMILY.

On this new interpretation of the family *Heterophyidae* the following sub-families and genera, which have been included in it by Nicoll and Poche, must now be excluded.

The genera united in the sub-family *Microphallinae* Ward, 1901, do not belong to the *Heterophyidae*, since they lack a seminal receptacle and some of them are provided with a cirrus pouch.

The species united in the sub-family *Gymnophallinae* Odhner, 1905, do not belong to the *Heterophyidae* because they lack a seminal receptacle.

The genus *Sigmaopera* Nicoll, in spite of its great resemblance to *Heterophyidae*, cannot be assigned to this family because of its well-developed cirrus pouch.

. The genus *Nanophyetus* Chapin (in Hall, 1927) is characterised by the presence of a well-developed cirrus pouch and does not possess a seminal receptacle (of this I convinced myself after examining the cotype specimens). It does not therefore belong to *Heterophyidae*.

The genera *Euryhormis* Poche, 1926, and *Taphrogonimus* Cohn, 1904, are based on insufficient descriptions of their representatives and therefore there is no sufficient reason for including them in the *Heterophyidae*.

The genera *Parabascus* Looss, 1907, and *Cryptotrema* Ozaki, 1926, certainly do not belong to *Heterophyidae*.

The genus *Paracoenogonimus* Katsurada, 1914, appears to be a synonym of the genus *Prohemistomum* Odhner, 1919, or *Cyathocotyle* Mühling, 1896, which do not belong to *Heterophyidae*.

The genus *Opisthometra* Poche, 1926, belongs to *Acanthochoasmidae*, but not to *Heterophyidae*.

The genus *Cladocystis* Poche, 1926, should be relegated to the *Opisthorchidae* until the genital pore of its members is re-described.

On the other hand, I include in *Heterophyidae* the genus *Stictodora* Looss, 1899, for which Poche has created an unnecessary family *Stictodoridae*, relying on the incorrect description of Looss, and four new genera: *Dexiogonimus*, *Diorchitrema*, *Cercarioides*, and *Adleria*.*

It is probable that some of the genera and sub-families which have been excluded from *Heterophyidae* in this paper will be assigned to the super-family *Opisthorchoidea* after further investigations. On the other hand, it is very probable that many known species which are at present included in other systematic groups will, after correct re-description, be assigned to the *Heterophyidae*.

METHOD OF CLASSIFICATION.

Since the above-mentioned genera are excluded from the family *Heterophyidae*, the remainder should be distributed in sub-families according to the method defined by Ciurea, i.e., according to the details of the structure of the genital pore. But this method has disadvantages, for in *Heterophyidae*, in contrast to other Trematode families, the position and the structure of the genital pore are very variable and proved to be valid only as generic characters. On Ciurea's method one should create almost as many sub-families as there are genera. Not the most changeable, but on the contrary, the most constant features should be taken as a basis for division into sub-families.

On investigating the comparative anatomy of known genera of *Heterophyidae* it appeared that they may be readily arranged in homologous rows, according to the principle of taxonomical coefficients, which I have introduced for *Cyclocoelidae* (1926).

The following complex of features was found useful for a sub-family coefficient, i.e., for characterising a sub-family.

(1) The shape of the anterior part of the body (dilated or not).

*The *Heterophyidae* appear to be most closely related to the *Opisthorchidae* Lühe and both form the nucleus of a new super-family *Opisthorchoidea*. They have a common scheme of anatomical structure, both in adults and cercariae, and similar life-histories. They differ from each other mainly in the structure of the terminal portion of the genital ducts and in that *Heterophyidae* parasitise the intestine while *Opisthorchidae* are found in the bile ducts of final hosts.

(2) The presence or absence of conspicuous spines round the oral aperture, and

(3) The number of testes and their position in relation to the ovary (in front or behind it).

The distribution of vitellaria may be regarded as a coefficient of a tribe, i.e., it serves to distinguish tribes.

Various combinations of the following characters form a generic coefficient: (1) the arrangement of the genital glands; (2) the structure and position of the ventro-genital sac; (3) the additional structures of the oral apparatus, and (4) where division into tribes is not indicated, the distribution of the vitellaria.

All other peculiarities are specific characters.

On this scheme all existing genera of *Heterophyidae* may be distributed among five sub-families: *Heterophyinae* Ciurea, 1924, *Centrocestinae* Looss, 1899, *Cercarioidinae* n. subf., *Haplorchinae* Pratt, 1902, and *Adleriinae* n. subf. These sub-families may be determined on the following key:

A. Testes two:

- (1) the anterior part of the body very dilatedCERCARIOIDINAE
- (2) the anterior part of the body not dilated
 - (a) circumoral spines presentCENTROCESTINAE
 - (b) circumoral spines absentHETEROPHYINAE

B. One testis:

- (1) ovary in front of the testisHAPLORCHINAE
- (2) ovary behind the testisADLERIINAE

Every sub-family could be divided into two tribes, according to the distribution of the vitellaria. In some genera the vitellaria are confined to the region behind the level of the ovary, in others they extend anteriorly beyond the genital aperture.

These features are observed in three of the five sub-families and they may therefore be considered as homologous. In the sub-family *Heterophyinae*, which has many genera, such a division may be useful, but in other sub-families, which contain comparatively few genera, division into tribes is not yet necessary. It is probable that when more genera are established, the division of all sub-families of *Heterophyidae* into tribes will be practical.

As pointed out, the sub-family *Heterophyinae* is the richest in genera and in it one observes the most diverse combinations of features, which form the generic taxonomical coefficient, i.e., all

possible arrangements of the genital glands and various structures of the ventro-genital sac.

The arrangement of the testes is the most noteworthy feature. One can recognise three main dispositions: (1) testes lying side by side, (2) obliquely to the long axis of the body, and (3) almost along this axis. In addition, they may be situated at the posterior extremity of the body or removed anteriorly; thus six dispositions of the testes exist. Each mode of arrangement of the testes may be represented by several genera which differ from each other in the position or structure of the ventro-genital sac and the distribution of the vitellaria.

All the above six dispositions of the testes are possible in other sub-families, but actually only some of them have been found so far.

If one arranges all the known genera of *Heterophyidae* in a table in which those belonging to one sub-family are placed in longitudinal rows and each space in the transverse direction corresponds to a definite disposition of the testes, one receives a table of homologous rows, in which the parallelism in the development of the features in different sub-families of *Heterophyidae* is clearly seen. (See Table I.)

Many empty squares remain in this table—they wait for still undescribed genera, the main features of which can easily be foretold.

LIST OF SPECIES

In the following list all species of *Heterophyidae* are arranged according to the new classification. (*—Denotes those found in Palestine.)

A. *Heterophyinae* Ciurea, 1924

(a) *Heterophyinae* n. tr.

I. *Diorchitrema* n. gen.

*(1) *D. pseudocirrata* n. sp.

II. *Dexiogonimus* n. gen.

*(2) *D. ciureanus* n. sp.

III. *Heterophyes* Cobbold, 1866

*(3) *H. aequalis* Looss, 1902

*(4) *H. dispar* Looss, 1902

*(5) *H. heterophyes* (Siebold, 1852)

(6) *H. nocens* Onji, 1915

IV. *Metagonimus* Katsurada, 1912

(7) *M. romanicus* (Ciurea, 1915)

(8) *M. yokogawai* (Katsurada, 1912)

TABLE I

Disposition of the testes	TWO TESTES				ONE TESTIS		
	<i>Heterophyinae</i>		<i>Centrocestinae</i>		<i>Cercarioiinae</i>		<i>Haplorchinae</i>
	<i>Vitellaria</i> short (<i>Heterophyrea</i>)	<i>Vitellaria</i> long (<i>Cryptocoryle</i>)	<i>Vitellaria</i> short	<i>Vitellaria</i> long	<i>Vitellaria</i> short	<i>Vitellaria</i> long	<i>Vitellaria</i> short <i>Vitellaria</i> long
Side by side	<i>Diorchitrema</i> <i>Doxigenimus</i>	<i>Cryptocoryle</i>	<i>Pygidioptis</i> <i>Parascocyle</i>	<i>Centrocestus</i> <i>Asococyle</i> <i>Stamnosoma</i>
	<i>Heterophyes</i> <i>Metagenimus</i>	<i>Tocotrema</i> <i>Rassicotrema</i>
	...	<i>Apophallus</i>
In the hindmost portion of the body
	Obliquely
	One behind the other
Removed from the posterior extremity of the body
	Obliquely
	One behind the other

- V. *Stictodora* Looss, 1899
 *(9) *S. sawakinensis* Looss, 1899
- VI. *Galactosomum* Looss, 1899
 (10) *G. lacteum* (Jägerskjöld, 1896)
 (11) *G. erinaceum* (Poirier, 1886)
- VII. *Microlistrum* Braun, 1901
 (12) *M. cochlear* (Diesing, 1850)
 (13) *M. cochleariforme* (Rudolphi, 1819)
 (14) *M. semifuscum* (Olsson, 1876)
 (15) *M. spinetum* (Braun, 1901)
- (b) *Cryptocotylea* n. tr.
- VIII. *Cryptocotyle* Lühe, 1899
 (16) *C. concavum* (Creplin, 1825)
 (17) *C. cryptocotyloides* (Issaitshikoff, 1923)
 (18) *C. quinqueangulare* (Skrjabin, 1923)
- IX. *Tocotrema* Looss, 1899
 (19) *T. (?) echinata* (Linstow, 1878)
 (20) *T. jejunum* Nicoll, 1907
 (21) *T. lingua* (Creplin, 1825)
- X. *Rossicotrema* Skrjabin, 1919
 (22) *R. donicum* Skrjabin, 1919
- XI. *Apophallus* Lühe, 1909
 (23) *A. mühlungi* (Jägerskjöld, 1899)
- B. *Centrocestinae* Looss, 1899
- XII. *Pygidiopsis* Looss, 1907
 *(24) *P. genata* Looss, 1907
- XIII. *Parascotyle* Stunkard and Haviland, 1924
 *(25) *P. ascolonga* n. sp.
 *(26) *P. italica* (Alessandrini, 1906)
 *(27) *P. longa* (Ransom, 1920)
 (28) *P. minuta* (Looss, 1899)
 (29) *P. nana* (Ransom, 1920)
 (30) *P. pithecophagicola* (Faust, 1920)
- XIV. *Centrocestus* Looss, 1899
 (31) *C. cuspidatus* Looss, 1899
- XV. *Ascocotyle* Looss, 1899
 (32) *A. coleostoma* Looss, 1899
 (33) *A. agrese* Travassos, 1916
- XVI. *Stannosoma* Tanabe, 1922
 (34) *S. armatum* Tanabe, 1922
 (35) *S. formosanum* Nishigori, 1924
- C. *Cercarioidinae* n. subf.
- XVII. *Cercarioides* n. gen.
 *(36) *C. abaronii* n. sp.
- XVIII. *Scaphanocephalus* Jägerskjöld, 1903
 (37) *S. australis* Johnston, 1917
 (38) *S. expansus* (Creplin, 1842)

D. *Haplorchinae* Pratt, 1902XIX. *Monorchitrema* Nishigori, 1924*(39) *M. taibokui* Nishigori, 1924*(40) *M. taibui* Nishigori, 1924XX. *Haplorchis* Looss, 1899(41) *H. cahirinus* (Looss, 1896)(42) *H. pumilio* (Looss, 1896)E. *Adleriinae* n. subf.XXI. *Adleria* n. gen.*(43) *A. minutissima* n. sp.IV. CLASSIFICATION AND LIFE HISTORY OF THE
*HETEROPHYIDAE*Sub-family *HETEROPHYINAE* Ciurea, 1924.

Diagnosis : *Heterophyidae* with more or less flattened body, more so anteriorly than posteriorly ; no dilation of the anterior extremity ; no circumoral spines ; two testes situated behind the other reproductive organs.

Type genus :—*Heterophyes* Cobbold, 1866.

This sub-family may be divided into two tribes—*Heterophyea* and *Cryptocotylea*.

Key to the genera of the sub-family HETEROPHYINAE.

- A. The vitellaria do not extend anteriorly beyond the level of the ovaryTribe *HETEROPHYEA*.
- I. The testes are situated at the posterior extremity of the body :
- (a) the testes lie side by side :
- (1) the ventro-genital sac is situated near the middle line of the body*Diorchitrema*.
- (2) the ventro-genital sac is situated near the right border.....*Dexiogonimus*.
- (b) the testes lie obliquely to the axis of the body :
- (1) the ventral sucker is separated from the genital sac and lies at the middle line of the body...*Heterophyes*.
- (2) the ventral sucker is included in the ventro-genital sac near the right border of the body.....*Metagonimus*.
- II. The testes are removed towards the middle of the body :
- (a) the testes lie obliquely :
- (1) the stem of the excretory bladder is S-shaped...*Stictodora*.
- (2) the stem of the excretory bladder is Y-shaped...*Galactosomum*
- (b) the testes lie one behind the other*Microlistrum*.

- B. The vitellaria extend anteriorly beyond the level of the genital aperture Tribe *CRYPTOCOTYLEA*.
- I. The testes lie side by side; the shape of the body is almost round *Cryptocotyle*.
- II. The testes lie obliquely to the axis of the body which is oval or pyriform :
- (a) one gonotyl in the ventro-genital sac *Tocotrema*.
- (b) there are two gonotyls guarding the entrance to the opening of the ventro-genital sac *Rossicotrema*.
- III. The testes lie one behind the other; the body is very elongated *Apophallus*.

Tribe *HETEROPHYEA* nov. tr.

Diagnosis: *Heterophyinae* in which the vitellaria do not extend anteriorly beyond the level of the ovary.

Type genus:—*Heterophyes* Cobbold, 1866.

Genus *HETEROPHYES* Cobbold, 1866.

A. HISTORY OF THE GENUS.

The genus *Heterophyes* Cobbold, 1866, is not only the first of the family *Heterophyidae*, but is also one of the first genera to be formed out of the mass of specific names of Trematoda, which were all included in the generic name *Distoma* until the end of the last century. It quickly attracted the attention of the investigators owing to a peculiarity of the genital apparatus characterised by the presence of a third so called 'genital sucker', which distinguished it from all hitherto known Trematoda.

Although the literature concerning this genus and its representatives is rather large, there exist few original works. Looss (1894 and 1902) provided descriptions of Egyptian species, and Japanese authors (1915 and 1926) described two species from the Far East. For the rest authors contented themselves with quotations or slight modifications of the original descriptions.

The first species of the genus *Heterophyes* was *Distoma heterophyes* Siebold, 1852, found in a man in Cairo during autopsy. This species has been subsequently referred to by several authors under the names: *Distoma heterophyes hominis*, *Dicrocoelium heterophyes*, *Fasciola heterophyes*, *Heterophyes aegyptiaca*, *Mesogonimus*

heterophyes, *Cenogonimus heterophyes*, etc. In 1900 Stiles gave it as his opinion that according to the international rules of nomenclature, the correct name of this species should be *Heterophyes heterophyes* (Sieb., 1852), as the genus *Heterophyes* Cobbold, 1866, had been established with this species as type.

The early descriptions of this species were short and very imperfect. The first attempt to give a detailed description with figures was by Looss (1894 and 1896). Looss described two species—*Distoma heterophyes* Sieb. from man, dog, cat, fox and *Milvus aegyptius*, and a new species *Distoma fraternum* from dog, cat and pelican.

Later (1902), Looss published a revision of the genus *Heterophyes* in which he pointed out that in his earlier papers several species had been described under one name. Looss reclassified his material on a new basis and created six species and two sub-species out of the first two species: (1) *H. fraternus* (Looss, 1894) sens. str. from pelican, (2) *H. inops* Looss, 1902, from pelican and *Milvus aegyptius*, (3) *H. aequalis* Looss, 1902, from dog and cat, (4) *H. dispar* Looss, 1902, from dog and cat, (5) *H. heterophyes* (Sieb., 1852) from man, dog and cat, (6) *H. pallidus* Looss, 1902, from *Milvus aegyptius*, (7) *H. heterophyes sentus* Looss, 1902, from dog and cat, and (8) *H. dispar limatus* Looss, 1902, from cats. The first four were described in his first papers under the common name *Distoma fraternum*, and the last four—under the common name *Distoma heterophyes*.

At the end of Looss's paper there is a note by Braun, adding a ninth species to the genus *Heterophyes*, namely *Cotylogonimus persicus* from a Persian wolf.

The genus *Heterophyes* was further enriched by two species from the Far East—*H. nocens* Onji, 1915, and *H. katsuradai* Ozaki and Azada, 1926. Thus, the number of species was raised to eleven.

However, in some recent works dealing with this genus the opinion was expressed that some species may be invalid and that their number should be reduced. In comparing the results of my own studies with the literature, I came to the conclusion that only three out of the described species may be considered as valid, namely:

1. *H. heterophyes* (Sieb.)
2. *H. dispar* Looss
3. *H. aequalis* Looss

H. nocens Onji requires further study to ascertain its validity, and the following are certainly not valid :

- | | | |
|-----|---|---|
| (1) | <i>H. heterophyes sentus</i> Looss |synonym of <i>H. heterophyes</i> (Sieb.) |
| (2) | <i>H. fraternus</i> Looss |" " " " |
| (3) | <i>H. pallidus</i> Looss |" " " " |
| (4) | <i>H. persicus</i> (Braun) |" " " " |
| (5) | <i>H. inops</i> Looss |" " <i>aequalis</i> Looss |
| (6) | <i>H. dispar limatus</i> Looss |" " <i>dispar</i> Looss |
| (7) | <i>H. katsuradai</i> Ozaki and Azada... |" " <i>nocens</i> Onji. |

In my study I compared the data in the literature, particularly the paper of Looss (1902), the most important of all, with the results of investigations on my own material and some cotypes. Professor J. Ciurea sent me a cotype of *H. heterophyes* obtained from Looss, Dr. L. Szidat sent the type specimen of *Coenogonimus persicus* and Dr. W. Arndt several bottles with *Heterophyes* species obtained from Looss. Unfortunately, the specimens from the Berlin Museum proved to be wrongly labelled and the collection could not, therefore, be used as a standard.

Although I have not had all the types at my disposal, my studies had this advantage, not enjoyed by other authors including Looss, that I used not only material from naturally infected animals, but also material from experimentally infected ones. Thus I had occasion to investigate the development of features, which are the most important for the differentiation of species, whereas to Looss there were accessible some disconnected stages of these features.

I propose the following new descriptions of species since I consider those of Looss to be based on an inevitably mistaken interpretation of characters. The diagnosis of the genus *Heterophyes* should also be re-defined.

'B. DIAGNOSIS OF THE GENUS *HETEROPHYES* COBBOLD.

Heterophyinae : Body tongue or pear-shaped ; the praepharynx and oesophagus well marked ; the ventral sucker situated near the middle of the body alongside the genital sac ; the testes are placed slightly obliquely to the axis of the body ; the ovary lies on the middle line in front of the testes ; the retort-like seminal receptacle lies behind the ovary ; the vitellaria lie behind the level of the ovary ; the uterus winds between the testes and the ventral sucker ; the seminal vesicle is divided into three parts separated from each

other by constrictions or short tubules ; the third part which is the smallest and has thick walls is the expulsor ; the male and female ducts unite before the genital aperture into a short ductus hermaproditicus which opens on the tip of the gonotyl ; the latter is a protrusible body bordering on the ventral sucker on its left side and behind it ; when erected it has the shape of a mushroom standing on a wide and short leg ; at other times it is ring shaped ; the free border of the gonotyl is armed with a circlet of spines, or comb-shaped plates 25 to 87 in number, interrupted on the side adjacent to the ventral sucker ; the excretory bladder is generally Y-shaped with a short stem and branches ; it lies between the testes and the seminal receptacle ; each branch receives a main vessel which is formed at the level of the oesophagus by the union of several smaller excretory ducts.

The adults parasitise the small intestines of mammals and birds.

The type species :—*Heterophyes heterophyes* (Siebold, 1852).

C. LIFE HISTORY OF THE MEMBERS OF THE GENUS *HETEROPHYES*.

As has already been mentioned, one can at present distinguish four species of this genus : *H. heterophyes* (Siebold), *H. dispar* Looss, *H. aequalis* Looss, and *H. nocens* Onji, the latter being doubtful.

The life history of the representatives of the genus *Heterophyes* has not yet been studied completely. Only a fragment is known, namely the development of the metacercariae of *H. heterophyes* in the dog, described by Khalil (1923) and that of *H. nocens*, described by Onji and Noshio (1915).

According to these authors, the metacercariae both of the Egyptian and Japanese species are located in the muscles of fishes of the genus *Mugil*. Infection occurs when dogs are fed on these fishes. In short, the life history of the above species does not differ from that of species of allied genera of *Heterophyidae* hitherto described.

As the work of Onji and Noshio was not accessible to me and the publication of Khalil bears the character of a short preliminary report, I made a series of experiments in order to investigate the full life history of the three species of the genus *Heterophyes* found in Palestine. The dissections of the street dogs and cats in Jerusalem

showed that species of the genus *Heterophyes* are their most common parasites. It was therefore concluded that their intermediate hosts must be sought in fish sold in the market.

For the purpose of detecting which species of fish are carriers of the larvae I obtained young dogs which had been taken away from their mothers and brought up on milk and fed batches of these animals with different species of fish. Several dogs were left for control. After two weeks the dogs were sacrificed and examined. It was determined that each of the following eight species of fish, namely, *Mugil cephalus*, *M. capito*, *M. auratus*, *Epinephelus enaeus*, *Tilapia simonis*, *Lichia amia*, *L. glauca*, and *Barbus canus*, are carriers of all three species of metacercariae.—*H. heterophyes*, *H. dispar*, and *H. aequalis*.

Encysted metacercariae were found without exception in every specimen of mullet and usually in large number, irrespective of the size or age of the fish. In one instance over one thousand cysts were found in one gram of muscle of *Mugil cephalus*. In other species of fish the metacercariae are rare, and one, therefore, may conclude that the fishes of the genus *Mugil* are favourite hosts of the members of the genus *Heterophyes*. The cysts are distributed uniformly, chiefly in striped muscles, but also in heart muscle, connective and fatty tissue, while they were not found in the skin, gut walls, internal organs, fins, scales and gills.

The cysts of the three species of *Heterophyes* are so similar morphologically, that it is impossible to differentiate them. They lie between the muscle fibres in the centre of a spindle-shaped mass of fat globules (fig. 1, c). The above formations are of different size, a feature probably depending on age. There is no pigmentation of the adhering tissue. The cyst itself, when examined through the lens on a dark background, appears white. The cysts are round or slightly oval and vary in size from 0.13-0.26 mm. Every cyst is isolated from the surrounding fatty formation by a very thin homogeneous membrane originating from the tissue of the host.

The wall of the cyst proper consists of a thick (0.004-0.012 mm.) fragile shell and a thin inner membrane (0.001-0.002 mm.). The shell breaks readily under the coverglass, and the metacercaria is then delivered. On pressing the cyst the metacercaria often becomes entangled in the inner membrane, and becomes free only after further manipulation.

D. MORPHOLOGY OF THE METACERCARIAE.

The metacercariae, like the cysts enclosing them, are very similar in all three species. Slight differences are found in the size and the internal anatomy. I shall confine myself to the description of metacercariae of *Heterophyes heterophyes*, which is very typical.

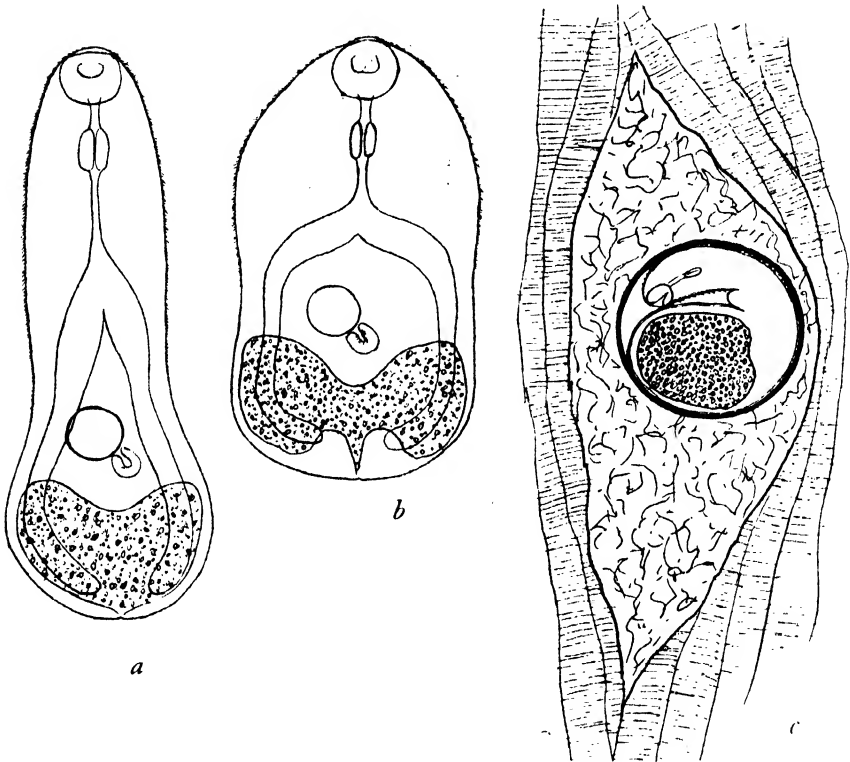


FIG. 1. Metacercariae of *Heterophyes heterophyes*.

The metacercaria, enclosed in the cyst is folded on itself (fig. 1, c) and continually moves round the centre of the cyst. The anterior part of the body is flattened, while the posterior one is round, and appears to be distended by the large excretory vesicle, which in some positions of the metacercaria occupies more than half of the cyst and has the appearance of a dark sac filled with very small refractile globules.

The larva, pressed out from the cyst is 0.21-0.40 mm. in length, moves slowly and shows large changes in shape (fig. 1, *a, b*). It lives only for a few minutes. When fully stretched, it is tongue-shaped, when fully contracted it is only half as long as in the retracted state and is heart-shaped. The first three-fourths of the body are thickly covered with scale-like spines. Numerous small masses of dark brownish pigment are scattered under the body surface.

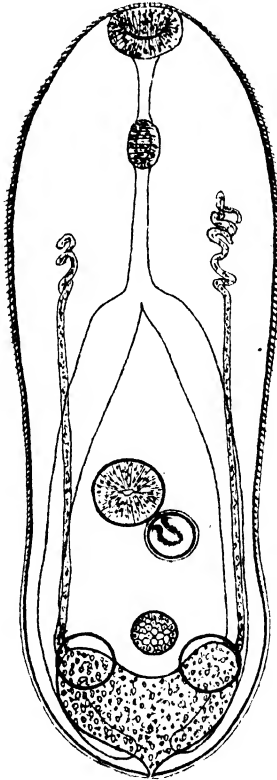


FIG. 2. One day old *Heterophyes heterophyes* from the dog.

In the living specimens one can make out only the suckers, gonotyl, pharynx and excretory vesicle, while in stained preparations the early stages of the genital glands are also seen.

The oral sucker measures 0.03-0.05 mm. ; praepharynx, 0.015-0.031 mm. ; pharynx, 0.028-0.031 mm. ; oesophagus, 0.05-0.11 mm.

Just behind the bifurcation the intestinal caeca are twice as wide as the oesophagus, but behind the ventral sucker they become much narrower; they reach the stem of the excretory vesicle. (In the metacercaria of *H. aequalis* they extend only up to the testes.)

The excretory vesicle is heart-shaped and occupies almost one-eighth of the body.

The ventral sucker is situated between the middle and the posterior third of the body; its diameter measures 0.03–0.04 mm., i.e., it is a little smaller than the oral sucker.

The gonotyl adheres to the left side of the posterior half of the ventral sucker. It is always retracted and is about 0.02 mm. in diameter. A horse-shoe shaped row of thickly arranged minute spines is present in the middle of the gonotyl.

The immature testes are spherical, about 0.03 mm. in diameter, being situated side by side in the posterior quarter of the body. They are usually covered by the excretory vesicle. The ovary is also spherical in shape, about 0.02 mm. in diameter and is situated on the middle line a little in front of the testes.

E. DEVELOPMENT OF HETEROPHYES IN THE FINAL HOST.

By feeding dogs with fish containing metacercariae and sacrificing some of them on successive days, one can easily follow the growth of the parasites and the development of their organs. It appears that all three species of *Heterophyes* develop similarly. The features peculiar to each species become evident during the first seven days. Only the largest metacercariae which are probably the only ripe ones develop into adults.

During the first two days after feeding, the anatomy of the young trematodes does not differ markedly from that of the metacercariae pressed out from cysts (fig 2). One day after the experimental feed they are already absent from the stomach and are found between the villi of the small intestine. Their posterior parts which are much thicker than the anterior ones project beyond the surface of the villi. It is not easy to detect them between the villi which they resemble in size and colour. I have found them under the microscope in scrapings of mucosa pressed between two glasses. The larvae are very motile and change their shape by contraction and extension (fig. 3).

After the end of the second day, the larvae already reach 0.34–0.50 mm. in size. The excretory vesicle varies from Y-shaped to heart-shaped, according to the amount of distension.

After the third day, the size of the body increases and there is a marked development of the inner organs. The amount of pigment decreases; the testes, round or oval (0.06–0.08 mm.), often separated from each other by the distended excretory vesicle, are seen posteriorly; in front of these the ovary is seen. The gonotyl in many specimens becomes protruded—probably a sign of the beginning fertilisation. The vitellaria begin to form.

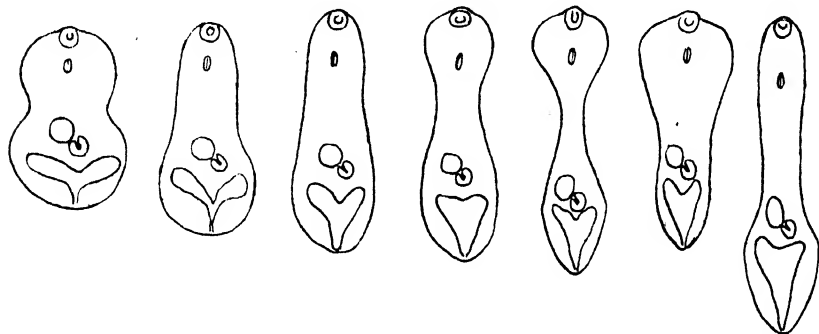


FIG. 3. Changes in shape during motion of two days old *Heterophyes heterophyes*.

On the fifth day the trematode is up to 0.8 mm. in length, the testes, 0.06–0.12 mm.; the ovary, 0.04–0.09 mm. In almost 10 per cent. of worms one or two transparent eggs are seen in the initial portion of the uterus.

On the sixth day the length of the body is between 0.8–1.1 mm.; the testes, 0.1 mm.; the ovary, 0.05–0.08 mm. The vitellaria are already well developed and the pigment has almost disappeared. There are eggs in all specimens, their number varying from 2 to 50. The majority of the eggs are still transparent, only the oldest being yellowish.

On the eighth day some specimens reach 1.3 mm. in length. The testes are 0.10–0.14 mm., the ovary 0.07–0.08 mm. The whole uterus is full of eggs which begin to escape; at this stage they can be detected in the faeces. The worms are more or less dark and are long enough to project beyond the surface of mucosa. They can be

seen with the naked eye and appear as small dark dots studding the surface of the mucosa.

I am not inclined to the view of Ciurea and Yokogawa, that young *Heterophyidae* are confined to the villi of the mucosa only for the first three days and afterwards escape into the lumen of the intestine. They are confined to the villi in their early stages, but even as adults the villi are their natural habitat. Their posterior ends project beyond the surface of the mucosa because of their larger size. The oral sucker is relatively smaller in adults than in the young stages and they tend to fall out easily and are, therefore, found in the intestinal contents.

Mature specimens are found mostly in the middle part of intestines but they are sometimes found equally distributed throughout the whole length of the small intestine.

F. REMARKS ON THE MORPHOLOGY OF ADULTS.

Looss, and authors who followed him, relied on certain characters to which they attributed specific significance, and on these characters they distinguished nine species and two sub-species. A careful study of hundreds of specimens belonging to three species, led me to the conclusion that most of these features depend on age, on merely individual peculiarities, or are due to fixation. The measurements of the body which Looss determined very carefully are only of relative importance. The living worms are very motile and, therefore the shape and size of their bodies depend on the amount of contraction at the moment of death. Worms which have been washed out from the intestine and allowed to die in cold water have a constant shape which does not change considerably on fixation in 70 per cent. alcohol. Material which is fixed alive in alcohol or formalin contracts, more so in formalin than in alcohol.

The dead worms found in the intestine of animals some time after the death of the latter are almost always found to be extended.

The shape of the internal organs, which are elastic, depends on the amount of contraction or extension of the worm (compare fig. 4 with fig. 5b). The enumeration of the vitelline follicles is also of limited importance for they vary in number and shape in every species.

These data were used by the previous authors because they were considered of specific importance. Looss also considered the relation

between the sizes of suckers and the colour of eggs to be of great specific importance. In spite of the fact that in his preface (1902) he pointed out that the suckers grow with age, he gave very precise measurements for every species, and further, he differentiated his two subspecies ('formen')—*H. heterophyes sentus* and *H. dispar limatus* on this character.

A study of the developing worms show that Looss's figures are irrelevant. For, in the very young *Heterophyes heterophyes* the oral sucker is the largest and the gonotyl is the smallest, while in the adults the conditions may be reversed. This is seen from the Table II, made from a series of specimens obtained from dogs.

TABLE II.

Showing the relation between sizes of oral sucker, ventral sucker and gonotyl of a series of specimens of *H. heterophyes* from the dog.

Length of the worm	Oral sucker	Ventral sucker	Gonotyl	Remarks
mm.	mm.	mm.	mm.	
0.40	0.046	0.046	0.034	1 day old
0.61	0.046	0.056	0.068	3 days old
0.83	0.056	0.093	0.115	4 days old
0.94	0.065	0.133	0.152	6 days old
1.39	0.074	0.229	0.232	24 days old
1.89	0.090	0.254	0.248	
1.98	0.118	0.316	0.310	

The colour of eggs to which Looss ascribed a great importance is of no value for the differentiation of species. In every tube containing worms of the same age there are all gradations in the colour of eggs, which vary from almost transparent to dark brown.

Specimens of *Heterophyes heterophyes* from *Circaetus gallicus* were exceptional in that they had almost colourless eggs, like those of *Heterophyes pallidus* Looss. It appears that the colour of the eggs does not depend on the species of the worm, but on its environment.*

* Barlow (1925) made the same observation for the eggs of *Fasciolopsis buski*. This phenomenon must have attracted the attention of all workers in the course of examination of faeces for eggs of Trematodes and Nematodes.

FIG. 1.

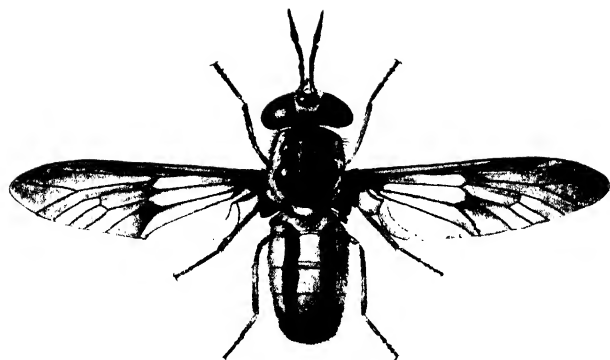


FIG. 2.

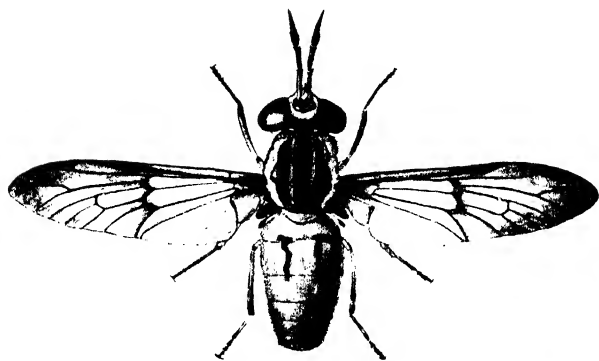


FIG. 3.



FIG. 1. ♀ *Chrysops dimidiata*.

FIG. 2. ♀ *Chrysops silacea*. E. M. Patton, del.

FIG. 3. Mouth parts of *Chrysops silacea*, shewing several mature embryos of *Loa loa* at the tip of

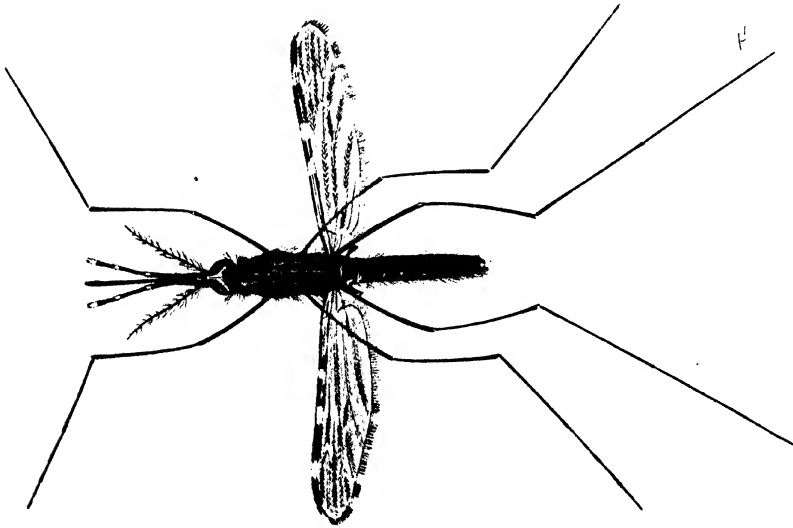


FIG. 1.
FIG. 1. ♀ *Anopheles culicifacies*. A. J. Engel Terzi, del.

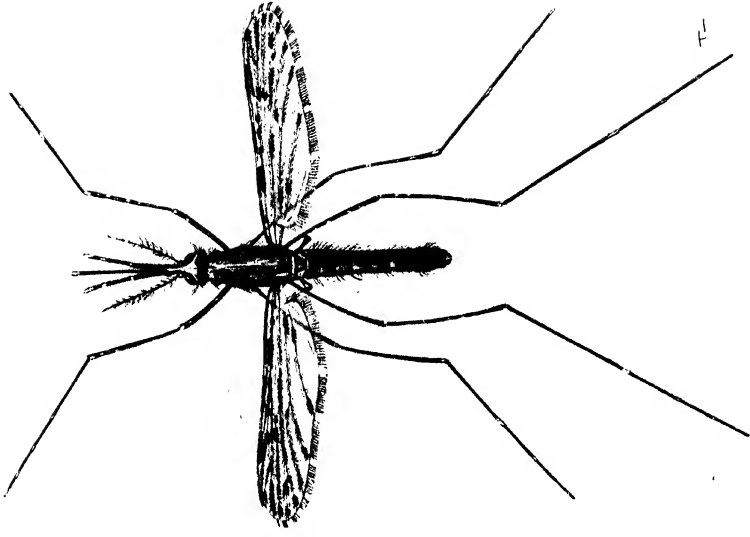


FIG. 2.
FIG. 2. ♀ *Anopheles stephensi*.

membrane, which in parts is thickened by circular muscles. Also note the longitudinal muscle bundle cut transversely; these occur at intervals. This section is through the dilated part of the mid intestine, which is usually known as the stomach. There is little or no difference to be observed between the cells lining the narrow part, the cardia, and those lining the stomach, except that those of the latter are slightly longer. The circular fibres are well marked at the lower end of the mid intestine, where they form a sphincter, which separates the mid intestine from the hind intestine.

Class Hexapoda

Order Diptera

Suborder Cyclorrhapha

Family Muscidae Calypteratae

Subfamily Muscinae

Genus *Musca*.

Specimen 69. Entire alimentary tract of

♀ *Musca domestica*, dissected out, fixed to a coverslip with Bles's fluid, stained with Delafield's hæmatoxylin, and mounted in Canada balsam on a slide. Fig. 95. Low power objective only.

As already noted, the alimentary tract in the higher Diptera is very long, and there is no dilated flask-shaped portion as in the lower Diptera. It is thus a more highly organized canal, and its increase in length and reduction in calibre is of great advantage to these flies, as it has a much larger absorptive surface, and the food is closer to the digestive cells. FORE INTESTINE. The fore intestine of the house fly consists of the prestomum between the labella where the pseudotracheal channels (collecting tubes, *see* fig. 205), lead into the food channel, formed by the labrum-epipharynx and hypopharynx; at the proximal end it is continued on through the pre and midpharynx (buccal cavity), into the pharynx, *ph.*, in the fulcrum, *fu*. It leaves it at the posterior end to become continuous with the œsophagus, *œ.*, the first part of which is dilated, but soon narrows and passes through the brain into the neck (*see* fig. 203, p. —); the structure of the wall is similar to the œsophagus of *Hæmatopota*, but it has more muscular fibres. It passes into the thorax, ventral to the *proventriculus*, *prov.*, which is a characteristic button-shaped structure, and is continuous with the duct of the œsophageal diverticulum, so that the fluid food of the fly, as it is sucked up into the fore intestine, passes straight on into the diverticulum. The œsophagus communicates with the proventriculus by a short duct which passes upwards and slightly backwards. CÆSOPHAGEAL DIVERTICULUM. *œ.dv.* The œsophageal diverticulum or crop, is a large thin walled sac, very similar to that of *Hæmatopota*, and is situated in the proximal part of the abdomen ventral to the mid intestine. Its walls are much thicker than those of the corresponding organ in the lower Diptera, the muscles and cells being distinctly more conspicuous. It always contains the food which the fly has been feeding on, and is commonly seen full of a yellowish fluid. Similarly, in the blood-sucking Muscinae, the blood always passes into the œsophageal diverticulum, and later is discharged into the mid intestine. This type of diverticulum is obviously

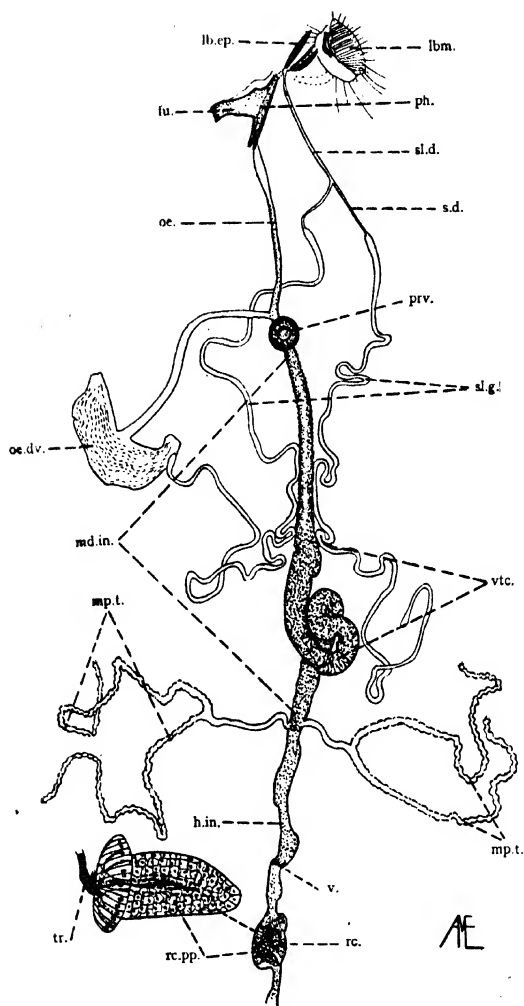


FIG. 95. Entire alimentary tract and salivary glands of ♀ *Musca domestica*. In such a dissection it is not possible to demonstrate the normal relations of the oesophagus and proventriculus; one rectal papilla is shewn enlarged. *fu.*, fulcrum; *h.in.*, hind intestine; *lb.ep.*, labrum-epipharynx; *lbm.*, labellum; *md.in.*, mid intestine; *mp.t.*, Malpighian tube; *œ.*, oesophagus; *œ.dv.*, oesophageal diverticulum; *ph.*, pharynx; *prv.*, proventriculus; *rc.*, rectum; *rc.pp.*, rectal papilla; *ph.*, pharynx; *s.d.*, duct of one salivary gland; *sl.d.*, salivary duct; *sl.g.*, salivary gland; *tr.*, trachea; *v.*, valve; *vte.*, ventriculus.

G. MORPHOLOGY AND CLASSIFICATION OF THE SPECIES OF THE GENUS *HETEROPHYES*.

Key to the Species.

- A. Less than 30 rodlets on the gonotyl:
- (1) the intestinal caeca end at the level of the anterior part of the testes..... *H. aequalis* Looss
 - (2) the intestinal caeca extend beyond the posterior borders of the testes..... *H. dispar* Looss
- B. Not less than 60 rodlets on the gonotyl:
- (1) not less than 62 rodlets on the gonotyl; Near East species..... *H. heterophyes* (Sieb.)
 - (2) not more than 60 rodlets on the gonotyl; Far East species *H. nocens* Onji

Heterophyes heterophyes (Siebold, 1852).

(Figs. 1-9).

I found this species in the dog, cat, Palestinian fox and in experimentally-fed rabbit and *Circaetus gallicus*. It is the largest of the three species under consideration. The shape of the body in non-contracted specimens depends on the host, being almost pear-shaped in the dog and tongue-shaped in *Circaetus gallicus* and the cat. The posterior part of the body is round or oval in cross section, and is slightly motile while the anterior part which is thinned dorso-ventrally and convex dorsally, is very motile.

The size depends on the host, worms of the same age being considerably smaller in the cat than in the dog.

The minimum size of a 7 days old specimen is 0.6 by 0.2 mm., the maximum size is 2.7 × 0.9 mm. in the dog and 1.3 by 0.3 mm. in the cat. The surface of the anterior two-thirds of the body is thickly covered with rows of scale-like spines, arranged diagonally. The spines are longest (up to 4μ.) at the level of the pharynx and become reduced in size posteriorly. The oral sucker is 0.05-0.18 mm., the ventral sucker 0.08-0.34 mm. in diameter. They vary according to the age of the worm. These variations are shown in Table II. The specimens from the cat have the ventral sucker comparatively a little smaller than those from the dog (fig. 5). In young specimens the ventral sucker lies on the boundary between the middle and posterior third of the body, in the adult it moves towards the middle of the body.

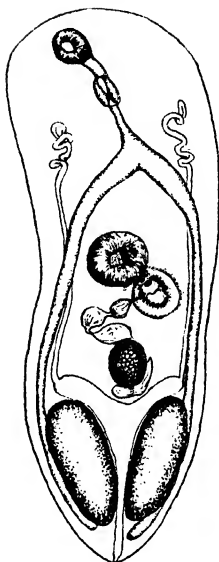


FIG. 4. Diagram of a moving specimen of *Heterophyes heterophyes* from a cat (the uterus not drawn).

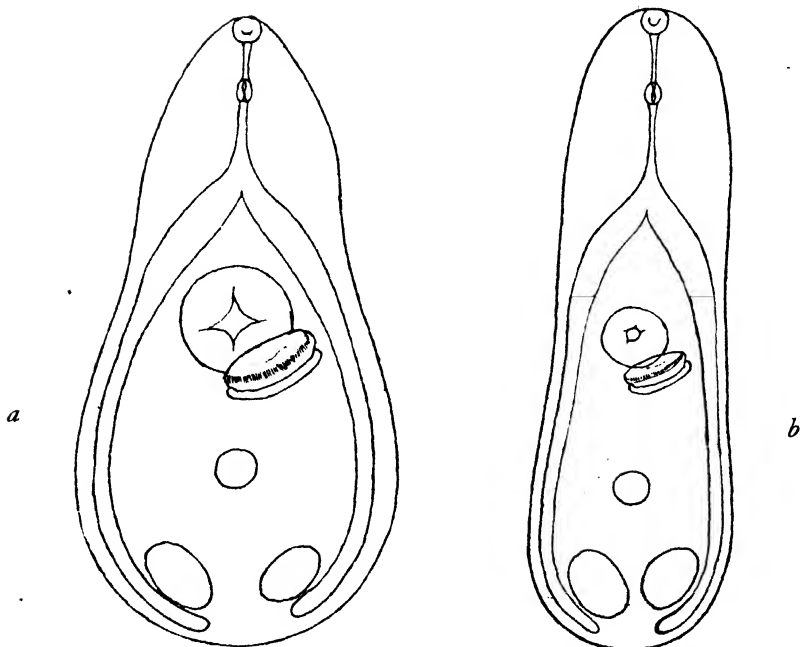


FIG. 5. Semi-diagrammatic outlines of two typical specimens of *Heterophyes heterophyes*: (a) from the dog, (b) from the cat, showing differences in shape.

The pharynx is oval, being 0.03–0.06 mm., the praepharynx 0.03–0.15 mm., oesophagus 0.08–0.43 mm. in length. The intestinal bifurcation lies on the boundary between the anterior and the middle thirds of the body. The caeca are widest at the level of the ventral sucker and are half as wide at the bifurcation and at the ends. They reach the extremity of the body where they turn round behind the testes. One caecum is often a little shorter than the other.

The testes are usually oval, but sometimes globular and are situated in the extreme hind part of the body. They lie usually at the same level, but the left testis is often situated a little anterior to the right one. Their long axes are not parallel but form an angle opening anteriorly. The right testis is usually somewhat larger than the left one, their diameter varies between 0.05 mm. and 0.29 mm., according to size of the worm. The ovary is always globular in shape, 0.07–0.15 mm. in diameter and lies in the middle line in front of the testes. The testes and the ovary attain their maximum size within the first two weeks, and therefore in large and old specimens they appear relatively small.

A retort-shaped seminal receptacle, varying in size according to the amount of distension with spermatozoa, lies behind the ovary. The Mehlis' body (shell gland) is dorsal to the seminal receptacle.

The vitellaria consist of two bunches of irregular pear-shaped follicles, scattered in the parenchyma under the ventral and lateral surfaces of the body between the levels of the testes and ovary.

The vasa efferentia unite in front of the ovary and pass into two seminal vesicles, separated from each other by a short duct. The vesicles vary greatly in shape and size. The second seminal vesicle is connected by a short tubule with a small oval or pyriform expulsor.

The ends of the male and female genital apparatus unite a short distance before the genital aperture, which is situated on the tip of the gonotyl. The gonotyl lies behind and on the left side of the ventral sucker, and is usually protruded. It has the shape of a mushroom and often overlaps a half of the ventral sucker (fig. 8). In flattened preparations it has the shape of a round or oval disc. The diameter varies between 0.11 and 0.31 mm., depending on the size of the worm and the degree of contraction. When protruded it may be sometimes larger than the ventral sucker, when retracted it is usually slightly smaller. The border of the gonotyl is armed

with a row of comb-shaped plates, interrupted only in the part in contact with the ventral sucker. The number of the plates is 73-87, their length 0.012-0.018 mm. They lie in the derma and their 5-6 sharp dents project outwards. When the gonotyl is retracted, the plates are also retracted and they then present the appearance of a horse-shoe.

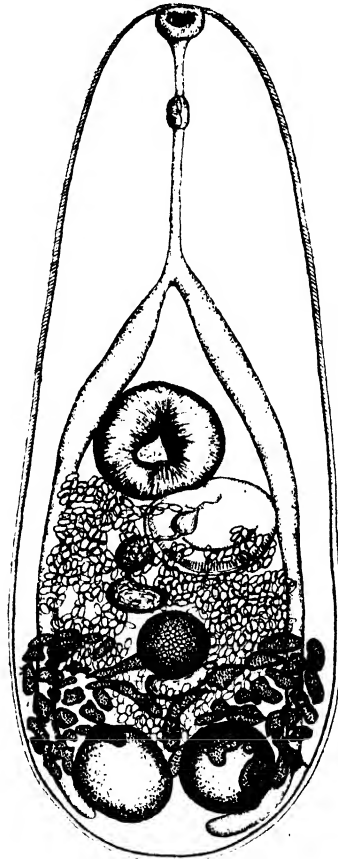


FIG. 6. *Heterophyes heterophyes* from the dog, from a slightly distended specimen (flattened preparation).

The uterus fills the whole free space between the testes and the ventral sucker and does not extend beyond these levels. The eggs are 0.023-0.027 mm. in length and 0.013-0.015 mm. in breadth ; they

may be transparent, or show various degrees of brown staining. Their shell is thick and sometimes shows distinct 'shoulders.' The measurements given by Looss—0.030 by 0.017 mm.—must be considered excessive.

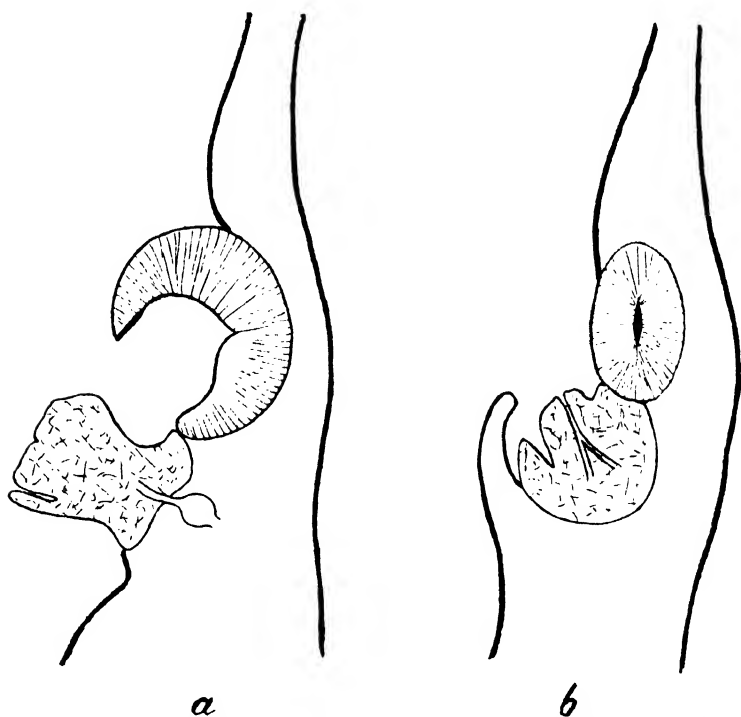


FIG. 7. Longitudinal section through ventral sucker and gonotyl of *Heterophyes heterophyes* showing: (a) the gonotyl protruded; (b) the gonotyl retracted.

I consider the following names as synonyms of *Heterophyes heterophyes*: *Heterophyes heterophyes sentus* Looss, 1902, *Heterophyes pallidus* Looss, 1902, *Heterophyes fraternus* Looss, 1902 and *Heterophyes persicus* (Braun) (other synonyms see in the reference list of the bibliography).

H. heterophyes sentus was found by Looss in cats and differs from the typical *H. heterophyes* in its somewhat smaller size and comparatively smaller ventral sucker. This resembles *H. heterophyes* (Sieb.) which I have always obtained from experimental cats. As dogs infected from the same material always contained typical

H. heterophyes, one must come to the conclusion that both species are identical, though in the dog they reach a greater size than in the cat.

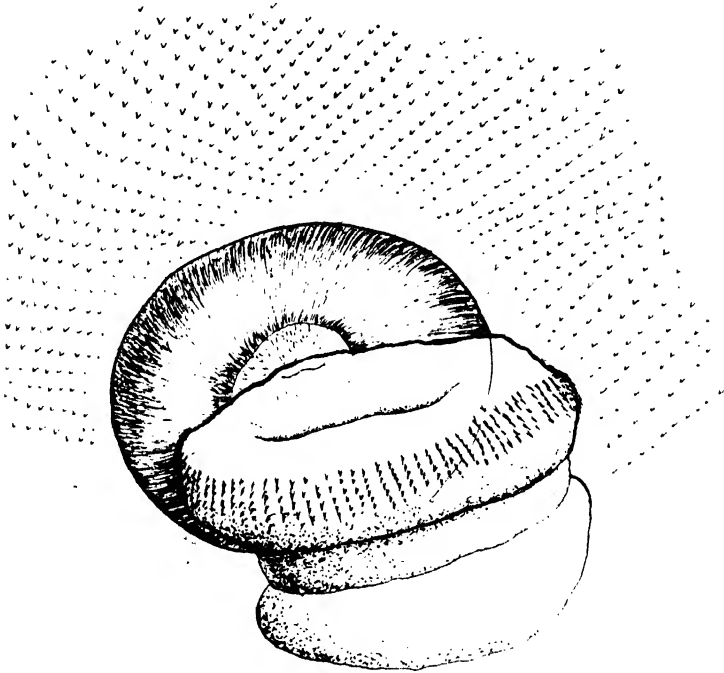


FIG. 8. Ventral sucker and protruded gonotyl of *Heterophyes heterophyes*.

Heterophyes pallidus, from *Milvus aegyptius*, has been described by Looss in his earlier works, as *Distoma heterophyes* Sieb. In his paper of 1902, he created this species obviously on the principle that similar species, parasitising birds and mammals, should be considered as distinct. However, this principle cannot be applied to *Heterophyidae*, since a considerable number of representatives of this family are known to be able to parasitise both birds and mammals (*Apophallus mühlingi*, *Pygidiopsis genata*, *Monorchitrema taihui*, etc.).

Heterophyes pallidus, according to the original description, differs from *H. heterophyes* only in having lighter eggs. The difference between these two species is even less than between *H. heterophyes*

and *H. heterophyes sentus*. It is evident, therefore, that *H. pallidus* is not a valid species and must be considered a synonym of *H. heterophyes*.

I found this 'species,' i.e., *H. heterophyes* with almost unstained eggs in *Circaetus gallicus*. This bird was caught young in the vicinity of Jerusalem and lived in the laboratory for some months. It was fed on meat, but occasionally fish (*Mugil* sp.) was given to it. As no examination of its faeces was previously made, one cannot affirm that it was infected with its trematode parasites in captivity. However, the fact that *H. heterophyes* is found in *Circaetus gallicus*, confirms the view that *H. pallidus* Looss is a synonym of *H. heterophyes* (Sieb.).

Among the material received from the Berlin Museum, there was a tube labelled *Heterophyes pallidus* Looss, from *Milvus aegyptius*, No. 4592. This sample proved to be typical *Heterophyes heterophyes* (Sieb.). Nevertheless, I cannot stress this as some of the tubes of this same collection appeared to be evidently wrongly labelled.

I consider *H. fraternus* as a synonym of *H. heterophyes*. The only description of this species is given in the paper of Looss published in 1902. In his earlier works he described under this name two different species—this and *H. aequalis*. The only drawing of this species given in Looss's work of 1896, apparently also represents mixed features of these species, and, therefore, no conclusions can be drawn from it. In any case, it does not correspond to the description given in 1902. In this description Looss pointed out that the anterior and posterior halves of the body do not differ in width, while on the drawing the body is distinctly pear-shaped; according to the description, the ventral sucker is considerably larger than the oral sucker—on the drawing they are almost equal.

On comparing Looss's description with my material of *Heterophyes heterophyes*, it is impossible to find any difference between them. I have had no type specimens. The tube from the Berlin collection labelled *Heterophyes fraternus*, No. 4591, proved to be identical with *Heterophyes dispar* Looss.

I also consider *H. persicus* (Braun) to be a synonym of *H. heterophyes*. I studied the type specimen of Braun kindly sent to me by Dr. L. Szidat and found no difference from *H. heterophyes*. It was an extended adult, measuring 2.54 mm. in length and

resembling in shape *H. heterophyes* as found in the dog. I also investigated the cotype specimens of this 'species' received from the Berlin Museum (No. 3935) and found them to be identical with the true *H. heterophyes*. They have been abnormally extended and denuded of spines, a sign that their host died long before they were removed from the intestine. The most extended specimens reached 3.8 mm. in length.*

Thus man, dog, Persian wolf, Palestinian fox, cat, rabbit, *Pelecanus onocrotalus*, *Milvus aegyptius* and *Circaetus gallicus* are all hosts of *H. heterophyes*. The dog appears to be the most suitable host, for the development of the parasite proceeds best in this animal. It is probably the chief reservoir of *H. heterophyes*.

Heterophyes nocens Onji, 1915.

I retain *H. nocens* provisionally. Some authors (Lane, Leiper, Faust) definitely consider it to be a synonym of *Heterophyes heterophyes*. However, Ciurea does not agree with them but holds it to be a valid species, and, still more, is of opinion that all *Heterophyes* described from Japan as *Heterophyes heterophyes* should be considered *Heterophyes nocens*.

On consulting the paper of Cort and Yokogawa, which contains the only description of this species in a European language, I came to the conclusion that the only feature that distinguishes this species from *Heterophyes heterophyes* is the number of rodlets on the gonotyl which in *H. nocens* does not exceed 60, but in *H. heterophyes* reaches 75-87. All the other characters mentioned by Cort and Yokogawa are unreliable for diagnostic purposes. Professor J. Ciurea has been kind enough to send me a preparation of *H. nocens*, sent to him by Professor Cort. Unfortunately I was not able to count the number of rodlets on the gonotyl and I was not able to recognise any feature that would distinguish it from *H. heterophyes*. According to its general appearance, this specimen was markedly contracted (perhaps during fixation). Therefore, the intestinal bifurcation was drawn nearer to the ventral sucker whilst the gonotyl was retracted and a little smaller than the ventral sucker. The eggs in the above

* In the same bottle I also found *Heterophyes dispar* Looss, *H. aequalis* Looss, *Parascocotyle longa* (Ransom), and *Pygidiopsis genata* Looss.

specimen measured 0.025 by 0.015, i.e., like those of *Heterophyes heterophyes*, and not in accordance with the original description (0.028 by 0.015).

The second Japanese species, *Heterophyes katsuradai*, recently described by Ozaki and Azada, seems to be identical with *H. nocens*. In distinguishing this species, the authors did not take into consideration individual variations and the influence of fixation. Azada pointed out *inter alia*, that the "neck" of *H. katsuradai* with included organs is much shorter than in other species of the same genus. The author loses sight of the fact that his preparations had been fixed in formalin and, therefore, it is not strange that they were strikingly contracted. The vitellaria in *H. katsuradai* unite in front of the testes, but they often do so in *H. heterophyes*. The eggs in *H. katsuradai* are said to be smaller and darker than in other species—if compared with my data, they are not smaller, and their colour is of no importance.

Heterophyes dispar Looss, 1902.

(Figs. 9 and 10.)

I found this species in dogs, cats, and experimentally infected rabbits, as well as in the sample of Trematoda received from the Berlin Museum and labelled *Cotylogonimus persicus* from Persian wolf. Its size is almost three-quarters that of *H. heterophyes*. This difference became more evident on dealing with adult specimens rather than with young ones. (It was easy to distinguish all three species of *Heterophyes* in material obtained from experimental animals infected by a single feed.) As in *H. heterophyes*, the worms from cats are always smaller and more slender than those from dogs. According to age and host, the length of this species varies between 0.4–1.4, the breadth 0.2–0.4 mm. The specimens from the Persian wolf were abnormally extended and reached up to 2.1 mm. in length. The body is usually tongue-shaped or oval, the anterior part being thinned with the lateral edges somewhat bent inwards. The posterior part of the body is oval in cross section. Except for the posterior extremity the whole surface is covered with large scale-like spines, arranged in alternating rows. The spines are longest at the level of the intestinal bifurcation, becoming gradually smaller towards both ends and disappear entirely posteriorly. In young specimens

they are small and lie close to each other while in the adults they are separated by greater intervals than in *H. heterophyes*.

In internal anatomy this species is very similar to *H. heterophyes*. It differs mainly in the relative sizes of the ventral sucker and the gonotyl and in the number of rodlets on the latter organ.

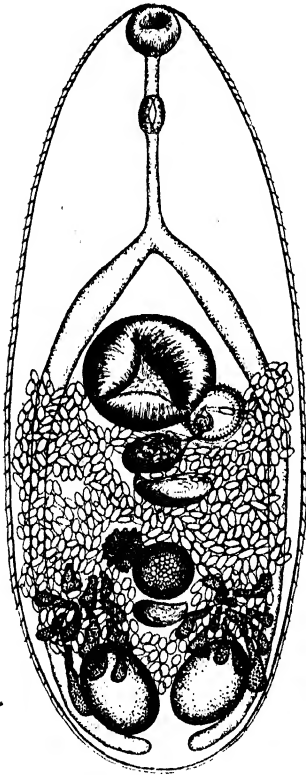


FIG. 9. *Heterophyes dispar*, adult specimen from the dog.

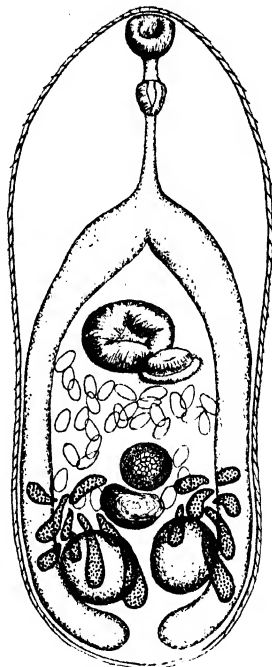


FIG. 10. Young specimen of *Heterophyes dispar* from the cat.

In adult specimens the oral sucker reaches 0.03–0.08 mm., the ventral one 0.05–0.25 mm. in diameter; the length of the praepharynx is 0.01–0.06 mm., of pharynx 0.03–0.04 mm., of oesophagus 0.08–0.12 mm. The intestine is similar to that of *H. heterophyes*, i.e., the limbs arise from the oesophagus very wide and become twice

as thin behind the ventral sucker ; in the posterior end of the body they bend behind the testes.

The globular or slightly oval testes are arranged almost side by side in the posterior part of the body, the left one being sometimes slightly in front of the other. The size of the left one is 0.04–0.15 by 0.04–0.12 mm., of the right one 0.05–0.18 by 0.04–0.14 mm. The ovary is round, 0.03–0.09 mm. in diameter. A small bean-shaped seminal receptacle varying in size according to the degree of distension with spermatozoa lies behind the ovary. The Mehlis' body lies usually to the right of the ovary.

The vitellaria consist of two bunches of follicles of irregular elongated pyriform shape, scattered in the lateral fields of the parenchyma between the levels of the ovary and testes.

The termination of the male genital ducts is typical of the genus, i.e., it consists of two seminal vesicles, separated from each other by a short constriction and from the small expulsor by a short duct.

The uterus fills the whole free space between the testes and the ventral sucker. Its end unites with the ductus ejaculatorius just before the genital aperture to form a short common duct which opens on the tip of the gonotyl. The latter adheres to the left side of the hind part of the ventral sucker. Its diameter does not exceed half that of the ventral sucker. Its border bears a row of thin spines, 25–30 in number, which is interrupted in the line of contact with the ventral sucker. Each spine is provided with two or three minute rodlets which are directed outwards.

The oval thick-shelled eggs are not distinctly narrowed at one pole like those of *H. heterophyes* and they are usually stained brown. Their size is 0.021–0.023 by 0.013–0.015 mm.

Heterophyes dispar appears to be the only species which has no synonyms. I had Looss's specimens of this species from the Berlin Museum at my disposal (No. 4591 and No. 4615), but they were wrongly labelled. Its hosts are dog, cat, Persian wolf and rabbit, but it may, like *H. heterophyes*, subsequently be found to parasitise other animals

Heterophyes aequalis Looss, 1902.
(Fig. 11).

I found this species in dog and cat as well as in the material from the Persian wolf. It is the smallest of the known species of the genus; its size being 0.4–0.9 by 0.2–0.4 mm. The body is pear-shaped or oval. The anterior part is somewhat flattened dorso-ventrally, while the posterior one is round in cross section. Except for the extreme hind part, the whole surface of the body is covered with scale-like spines, arranged thinly like those of *H. dispar*.

The oral sucker is 0.04–0.06 mm. in breadth, the ventral one 0.04–0.10 mm. Praepharynx 0.02–0.05 mm., pharynx 0.02–0.04 mm., oesophagus 0.06–0.11 mm. in length.

The intestinal limbs are four to five times as wide as the oesophagus and their width is usually uniform. They reach the level of the testes and never proceed beyond them.

The testes are nearly always globular and rather similar in size, measuring 0.05–0.11 mm. in diameter. They lie almost side by side in the posterior end of the body. When small they are separated by the trunk of the excretory vesicle, when large they press against each other.

The vasa efferentia open together into the hind seminal vesicle, the latter is separated by a short constriction from the second seminal vesicle which unites by a short tubule to the minute expulsor. The short but elastic ductus ejaculatorius unites with the mouth of the uterus to form a short common duct, opening on the tip of the gonotyl.

The ovary lies on the middle line somewhat in front of the testes. It is globular, measuring 0.04–0.07 mm. in diameter. Behind it lies a small bean-shaped seminal receptacle. The vitellaria consist of about 10 to 16 large pear-shaped follicles, grouped radially round the ovary. They seldom extend anteriorly beyond the front of this organ or beyond the intestinal limbs.

The uterus fills the whole space between the testes and the ventral sucker and opens into the common genital aperture.

The gonotyl is usually protruded and has the appearance of a disc 0.04–0.09 mm. in diameter, i.e., it is somewhat smaller than the ventral sucker. Its border bears a circlet of 15 to 25 thin spines, interrupted at the point of contact with the ventral sucker. The

eggs are oval, 0.023–0.025 mm. long and 0.014–0.016 mm. wide, with thick shells stained yellow or brown.

Looss also described *Heterophyes inops* from the pelican and *Milvus aegyptius* in the paper in which he described *H. equalis*. In comparing these two descriptions one is forced to the conclusion that they refer to one and the same species; 25 to 35 spines on the gonotyl, caeca extending up to the anterior part of the testes, are their common and very characteristic features. All the remaining

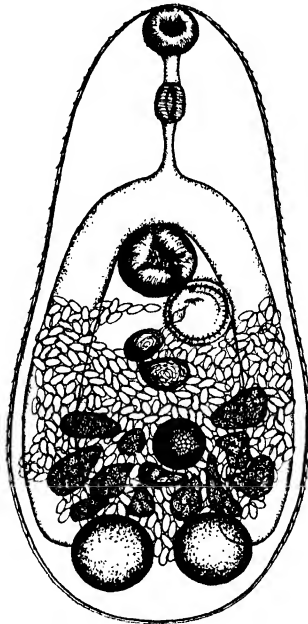


FIG. 11. *Heterophyes aequalis*, from the dog.

differences such as the size of the body, of the suckers, etc., indicates rather the age or the degree of contraction of the worm, but cannot be considered as specific characters.

It is evident that there are no anatomical differences between *H. aequalis* and *H. inops* but only difference in host such as exist between *H. heterophyes* and *H. pallidus*. Probably here, too, Looss distinguished these two species because one parasitises birds, and the second mammals. Thus there is no doubt that *H. aequalis* and *H. inops* refer to one and the same species. The question arises,

which of the two names is valid. I think that *H. aequalis* should be retained because under this name Looss described the parasites of carnivora, which are their main hosts, for in them they develop best.

The dog, cat, Persian wolf, pelican and *Milvus aegyptius* should be considered as hosts of *H. aequalis*.

In a tube from the Berlin collection, labelled No. 4614, there were three specimens of this species from a cat from Cairo. Their sizes are 0.34-0.49 by 0.17-0.18 mm. However, the glass No. 4615 labelled '*Heterophyes inops* Looss, Felis dom. Cairo' proved to contain specimens of *H. dispar* Looss. (The label is obviously wrong because Looss recorded '*H. inops*' only from the pelican and *Milvus aegyptius*, but not from the cat.)

H. OCCURRENCE OF HETEROPHYES SPECIES.

After feeding dogs and cats with suitable fish I invariably found all the three above-described species of *Heterophyes*. Under natural conditions these species do not occur with equal frequency. In dogs in Jerusalem *Heterophyes heterophyes* is found in 70 per cent., *H. dispar* in 40 per cent., *H. aequalis* in 20 per cent., while in cats respectively 80 per cent., 40 per cent., and 30 per cent. Cats are thus infected more frequently than dogs. This is explained by the fact that refuse bins are more accessible to cats than to dogs.

In all cases of the occurrence of the representatives of the genus *Heterophyes* in the above mentioned animals *H. heterophyes* is always found to predominate. *H. dispar* never occurs alone, but only as accompanying the former, and in numbers not exceeding 5 per cent. *H. aequalis* again does not occur alone, but only in the presence of two former species, not exceeding 1 per cent. of the number of *H. heterophyes*. This correlation was found both in dogs and cats in experimental and in natural infections. All three species occur together only in heavy infections. The number of worms found in natural infections varies from a few to several thousands. In an experimental dog, fed several times with mullet, I counted over 13,000 specimens of these parasites.

There is only scanty information as to the occurrence of *H. heterophyes* and the Japanese species in man. The former has been found several times in Egypt, while the eggs of the latter are common

in men (over 18 per cent.), in some districts of Japan. In Jaffa and Jerusalem the ova of *Heterophyes* are often found in faeces of man, examined in the laboratories of the Hadassah Medical Organisation. However, one must accept this date with reserve because other *Heterophyidae* such as *Dexogonimus*, *Monorchitrema*, etc., possess eggs which are similar to those of *Heterophyes* and can easily be mistaken for them. These remarks apply particularly to Palestine, where fourteen species of *Heterophyidae* occur.

Below, descriptions of other Palestinian *Heterophyidae* are given. Some of them have already been described by other authors but my researches revealed some differences (often considerable) from the existing descriptions. It is probably due to the mode of investigation and particularly to the method of fixation of the material. The following descriptions are based on the investigation partly of living material and partly of mounted preparations of specimens which were allowed to die in water and directly fixed in 70 per cent. alcohol. For elucidation of morphological details, serial sections were made.

The life-history of each species is not given, for in all cases it resembles that of *Heterophyes heterophyes*. In all species the life-cycle of which has been studied experimentally, the metacercariae are similar in appearance; they are encysted in the muscles of fishes and the adults begin to lay eggs on the eighth day after the infecting feed.

Genus *Metagonimus* Katsurada, 1912.

Up to the present three species of the genus *Metagonimus* have been described: *M. yokogawai* (Katsurada, 1912), *M. romanicus* (Ciurea, 1915), and *M. dobrogiensis* (Ciurea, 1915). Some authors presume that all these species are identical. I suggest that the two latter species are identical, for their original descriptions offer no sufficient grounds for separating them; the specific name *M. romanicus* should be retained for them.

According to the original descriptions there exist some small differences in the structure of the ventro-genital sac of *M. romanicus* and *M. yokogawai*. It is probable that these species are identical, but further comparative study is necessary before this point can be settled.

Genus *Dexiogonimus* n. gen.

This genus is closely related to the genus *Metagonimus* Katsurada from which it differs only in the position of the testes. The difference between these two genera might be compared to that between other genera of *Heterophyidae*, as for instance, *Apophallus* and *Rossicotrema*.

Diagnosis : *Heterophyinae*.—Body rounded posteriorly, tapered anteriorly, anterior part may be slightly wider than the posterior one; praepharynx and oesophagus well marked; the ventral sucker is modified, having a tubule-like slit instead of a cavity and is included in the ventro-genital sac; the testes lie side by side at the posterior extremity of the body; the large globular seminal receptacle lies in front of the right testis; the ovary lies in front of the testes on the middle line; the retort-like seminal vesicle may be divided by constrictions into several parts; there is no expulsor; the vitellaria are situated behind the level of the ovary; the uterine coils fill the free space between the levels of the testes and the ventro-genital sac and do not extend beyond these organs; the ventro-genital sac is situated near the border of the body, its opening being guarded by small muscular papillae; the excretory vesicle is Y-shaped with the stem as short as the branches. The adults are parasites of birds and mammals.

Type species :—*D. ciureanus* n. sp.

Dexiogonimus ciureanus n. sp.

(Figs. 12-17).

This specific name is given in honour of Professor J. Ciurea, who has made valuable contributions to the knowledge of the *Heterophyidae*.

Dexiogonimus ciureanus is frequently found in Palestinian dogs and cats, particularly in the neighbourhood of Lake Tiberias, where it is very common. I also found two specimens in an undetermined *Larus* sp. from the same locality. The second intermediate hosts are: *Tilapia simonis*, *T. galilea*, *Barbus canus*, *Discognathus* sp., *Mugil cephalus*, *M. capito*, *Lichia glauca*, the first two being the main ones.

The body of normal specimens has a peculiar typical shape somewhat resembling the sole of a foot, narrow posteriorly and wide anteriorly. The anterior and posterior parts are separated by a slight constriction. The specimens fixed alive in formalin, e.g., very contracted, are almost pentagonal. The specimens from *Larus* sp. were pear-shaped and were remarkable for their small size. As this fish-eating bird harboured only two specimens of *Dexiogonimus* one may suppose that it is not a suitable host and that the Trematodes found were young specimens which would have been shortly passed out.

The length of the body is 0.7–1.3 mm., the maximal breadth 0.3–0.7 mm. The whole body, except the hindmost part, is thickly covered with spines, which are longest at the level of the intestinal bifurcation. The oral sucker is 0.05–0.09 mm. wide, the prae-pharynx 0.02–0.06 mm., the pharynx 0.03–0.05 mm., and the oesophagus 0.06–0.09 mm. long.

The intestinal bifurcation is situated on the boundary between the first and second fourth of the body. The caeca are three times as wide as the oesophagus. From the bifurcation they pass obliquely towards the margins of the body, the right one turns in front of the ventro-genital sac, the left one some distance behind it, i.e., they are asymmetrical. They both reach the level of the middle of the testes.

The testes lie at the hind border of the body. They are globular or oval with the long axis horizontal, 0.09–0.18 mm. in diameter. When small they are separated by the stem of the secretory vesicle, when large they are adjacent.

The ovary is globular, 0.07–0.13 mm. in diameter, and lies on the middle line in front of the testes, from which it is separated by some uterine coils. To the right and a little behind it lies the seminal receptacle, which varies in size, according to the amount of distension by spermatozoa. When fully distended it may be larger than the ovary.

The vitellaria are situated at the margins of the body between the levels of the testes and the ovary. They consist of 15 to 30 small irregular follicles on each side.

The uterus in ripe specimens fills the whole free space between the level of the testes and the genital aperture. Some distance

before the genital aperture it unites with the ejaculatory duct to form a hermaphroditic duct.

The vasa efferentia open into a voluminous seminal vesicle 0.3 mm. in length, variable in shape and often showing two or three constrictions. An ejaculatory duct arises from the seminal vesicle and unites it with the uterus.

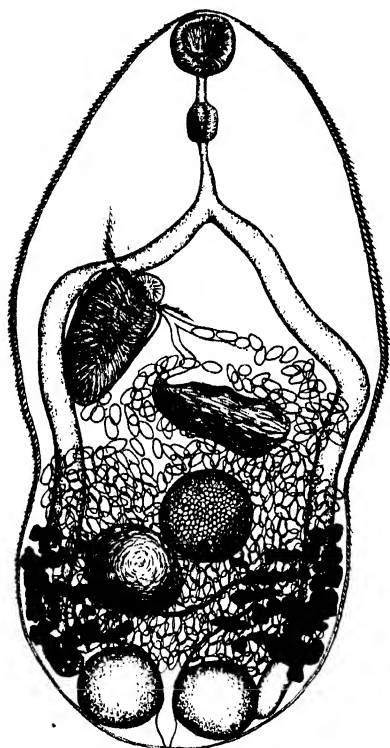


FIG. 12. *Dextogonimus ciureanus* from the dog, from a preparation fixed in alcohol.

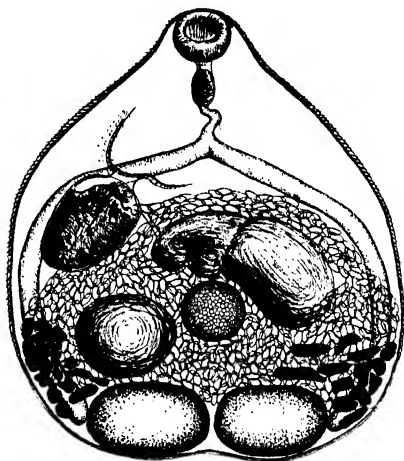


FIG. 13. *Dextogonimus ciureanus* from the same host as that of Fig. 12, after fixation in formalin.

There is no expulsor. The prostatic cells form a mass at the junction of the male and female ducts. The hermaphroditic duct formed by this junction opens on the median wall of the ventro-genital sac. The latter is situated near the right border of the body at the level of the junction of its first and middle third. It is an elongated depression directed somewhat obliquely to the axis

of the body and occupied by a large oval muscular body which is a modified ventral sucker. The latter may be completely retracted into the sac or its apex may protrude for a short distance.

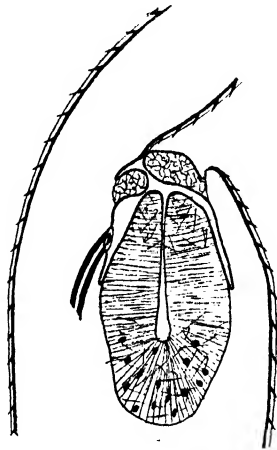


FIG. 14. Longitudinal section through the ventro-genital sac of *Dexiogonimus ciureanus* (semi-diagrammatic).

An oval transversely muscular papilla projects from the median border of the ventro-genital sac closing its aperture. Another muscular papilla is present on the dorsal wall of the sac just behind the former. The genital aperture lies behind the dorsal papilla—a condition which appears similar to that found in *Metagonimus yokogawai*. Both papillae together may be considered as a peculiarly modified gonotyl.

The eggs are oval with the anterior pole somewhat narrower than the posterior, their length is 0.025–0.028 mm., their width 0.015 mm.

Genus *Diorchitrema* n. gen.

Diagnosis : *Heterophyinae*—Body pear-shaped, the praepharynx and oesophagus well-marked ; globular ventral sucker included in the genital sac ; the testes lie side by side at the posterior extremity of the body ; the ovary in front of the right testis ; the globular seminal receptacle in front of the left testis ; the single small seminal vesicle is connected with a large expulsor ; the vitellaria confined

behind the level of the ovary ; the uterine coils do not pass beyond the levels of the testes and ventro-genital sac. The ventro-genital sac is situated near the middle line and contains no gonotyl. Adults are parasites of mammals.

Type species :—*D. pseudocirrata* n. sp.

Diorchitrema pseudocirrata n. sp.

(Fig. 15).

The representatives of this species are rare parasites of dogs and cats in Palestine. Their second intermediate hosts are fishes of the genus *Mugil*. They are small worms, reaching 0.3–0.6 mm. in length and 0.2–0.3 mm. in breadth. The body is almost round in cross section, the anterior portion being narrower than the posterior one. The whole body, except the most posterior part, is covered with small spines.

The oval sucker is 0.04–0.05 mm. in diameter ; the praepharynx is 0.01–0.04 mm., the pharynx 0.03–0.04 mm., the oesophagus 0.07–0.14 mm. in length. The intestinal bifurcation is situated in front of the middle of the body ; the intestinal caeca are equal, some times thicker than the oesophagus and reach the anterior borders of the testes.

The testes lie at the same level at the posterior border of the body. They are globular or slightly elongated and measure 0.06–0.12 mm. in diameter. When they are small they are separated from each other by the stem of Y-shaped excretory vesicle, but usually they are large and adjacent.

The globular ovary, 0.03–0.05 mm. in diameter, lies in front of the testes to the right of the middle line. To the left and a little behind it lies the seminal receptacle, which, according to the amount of distension, may be larger or smaller than the ovary.

The vitellaria consist of 20 to 40 large elongated follicles dispersed between the dorsal surface of the body and the testes.

The vasa efferentia open into a small, usually globular seminal vesicle, 0.018–0.037 mm. in diameter, connected by a short tubule with the expulsor. The latter is oval and relatively very large, being 0.07–0.10 mm. long and 0.04–0.06 mm. wide, with very thick walls in which spiral fibres are clearly seen. It lies in the middle

portion of the body at the left side and is oblique to the long axis of the body. A short ejaculatory duct arises from the expulsor and unites with the end portion of the uterus. The junction of the uterus and ejaculatory duct is surrounded by a mass of prostatic cells.

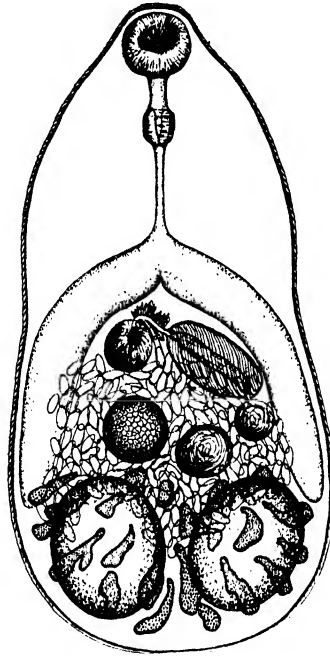


FIG. 15. *Diorchitrema pseudocirrata*, from the dog.

The uterus in adult specimens fills the whole free space between the expulsor and testes. It joins the ejaculatory duct to form a short hermaphroditic duct opening into the genital aperture. The latter opens on the dorsal wall of the ventro-genital sac at the base of the small ventral sucker, 0.03–0.04 mm. in diameter. The ventro-genital sac lies to the left of the middle of the body.

The eggs are oval, 0.018–0.021 mm. in length and 0.009–0.012 in width.

Genus *Stictodora* Looss, 1899

This genus was created by Looss for the species *S. sawakinensis* Looss, 1899 found in a sea-gull. Owing to the imperfect interpretation of the structure of the terminal portion of the male duct this genus could not be included in any group of Trematodes and for this reason Poche created for it a special family—*Stictodoridae*.

Examination of a cotype received from the Berlin Museum (No. 4594) and the material obtained from Palestinian animals both infected naturally and experimentally proved its relation to the *Heterophyinae*.

The diagnosis of the genus *Stictodora* may be modified as follows: Body elongated; both praepharynx and oesophagus well marked; ventral sucker absent; the testes lie in the middle of the hind part of the body obliquely to its axis, the ovary in front of the right testis, the globular seminal receptacle between the testes; the seminal vesicle consists of two parts divided by a constriction; it unites with a small expulsor. The vitellaria are confined to the posterior third of the body; the uterus coils between the genital aperture and the posterior extremity of the body; the genital sac is filled by a large protrusible gonotyl armed with spine-like plates; the genital aperture opens at the base of the gonotyl; the excretory duct is Y-shaped with the stem as short as the branches. Adults parasitise mammals and birds.

Type species:—*S. sawakinensis* Looss, 1899.

Stictodora sawakinensis Looss, 1899.

(Figs. 16-18).

Looss found this species in a *Larus* sp. and referred it to the family *Monostomidae*. In his description he reported the presence of a cirrus pouch, containing a small pars prostatica, ejaculatory duct and a thick armed (spinous) cirrus. Looss mistook the peculiarly constructed ventro-genital sac of this Trematode for the cirrus pouch.

Stictodora sawakinensis is found rarely in dogs and cats in Palestine and always in small numbers. I found it once in a bird—*Puffinus kuhli*, brought from Suez. The specimens of *Stictodora*

sawakinensis from the bird were considerably smaller than those from mammals.

The second intermediary host of *S. sawakinensis* in Palestine are fishes of the genus *Mugil*.

Body oblong, in normal state of distension and divided by a slight constriction into two parts—a short and narrow anterior one and a longer and wider posterior one.

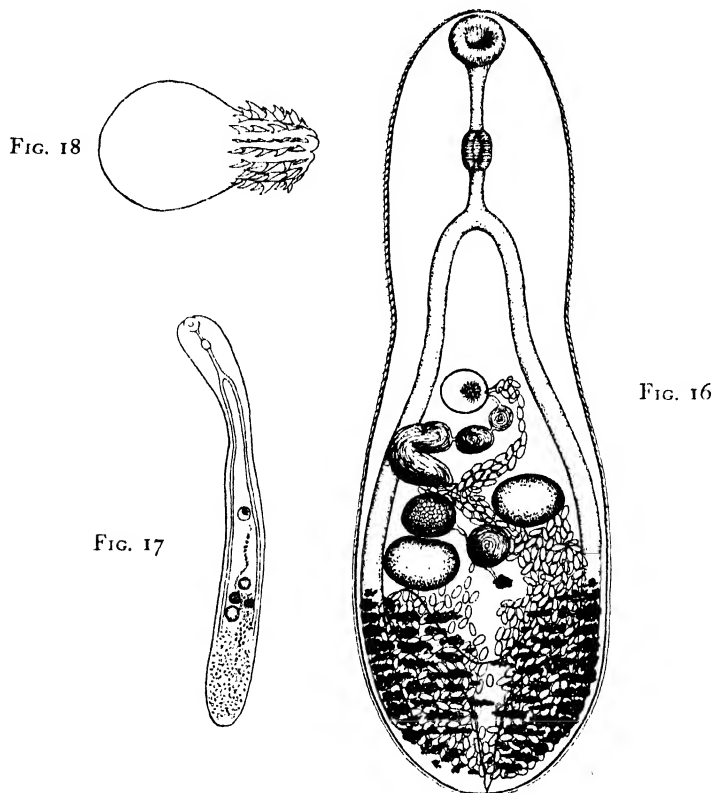


FIG. 16. *Stictodora sawakinensis*, from the dog.

FIG. 17. *Stictodora sawakinensis*, from *Puffinus kubli*.

FIG. 18. The gonotyl of *Stictodora sawakinensis* in the state of erection.

The anterior part is flat and concave like a spoon, while the posterior one is oval in cross section. The very contracted specimens are pear-shaped, with both extremities rounded, distended specimens are tongue-shaped.

The length of the body is 0.6–1.4 mm., the maximum width 0.2–0.4 mm. The oral sucker is 0.05–0.07 mm. in diameter, the praepharynx 0.04–0.09 mm., the pharynx 0.04–0.06 mm., the oesophagus 0.04–0.08 mm. in length.

The intestinal bifurcation in contracted specimens lies on the boundary between the first and second fourths of the body. The caeca are as wide as the oesophagus, straight, and reach almost to the posterior extremity of the body.

All the reproductive organs except the vitellaria are grouped in the third fourth of the body. The testes are round or transversely oval, 0.06–0.10 mm. in cross section and lie at an angle to the middle line, the left one in front of the right one. Between them lies an almost round seminal receptacle of varying width. The ovary is round, 0.05–0.08 mm. in diameter and lies, in normal specimens, in front of the right testis, but in stretched out specimens it lies between the testes while the seminal receptacle is pressed behind the left testis.

The vitellaria consist of rather small follicles arranged in transverse rows in the hind fourth of the body; the largest follicles lie near the border, the smallest near the middle line.

The vasa efferentia open into a large elongated and curved first seminal vesicle, which lies in front of the left testis. This vesicle is connected with a second smaller one, which is united by a short duct to a small expulsor from which the ejaculatory duct arises.

The uterus after leaving the seminal receptacle proceeds by a winding course towards the hind extremity of the body along the right side, turns forward, passes to the left testis, proceeds between it and the seminal receptacle and reaches the genital aperture. A little before its termination it may dilate up to 0.07 mm. in width to form a small egg receptacle usually filled with eggs.

The male and female ducts open side by side on the median wall of the genital sac.

In total preparation the genital sac has the appearance of a ring 0.04–0.07 mm. in diameter lying on the right side and a little in front of the middle of the body. The whole volume is occupied by a pear-shaped gonotyl which has a globular base and a cone-shaped retractile appendage. When contracted, the appendage is

totally withdrawn into the globular base, when extended it protrudes above the surface of the body in the form of an angular cone covered with six to ten longitudinal rows of triangular plates, 0.015 mm. long. There is no ventral sucker either in the genital sac or outside it.

The eggs practically fill the whole posterior quarter of the body and are oval in shape with thick shells stained dark brown. Their length is 0.027-0.030 mm., the breadth 0.015-0.017 mm.

The excretory vesicle is Y-shaped, its branches being as long as the stem. The right branch is adjacent to the right testis.

Genus *Galactosomum* Looss, 1899.

Two species may be assigned to this genus, *G. erinaceum* (Poirier, 1886) and *G. lacteum* (Jägerskjöld, 1896). Both are insufficiently described and it is not unlikely that when their descriptions will be complete they will find another position in the sub-family. As far as may be concluded from their actual descriptions, the genus *Galactosomum* is closely related to *Stictodora* and differs from it mainly in the structure of the excretory bladder which is S-shaped and not Y-shaped.

Genus *Microlistrum* Braun, 1901.

This genus was created by Braun to include three species: *M. cochlear* (Diesing, 1850), *M. cochleariforme* (Rudolphi, 1819) and *G. spinetum* Braun, 1901. Braun has misinterpreted the structure of the copulatory apparatus of these species, and this was corrected by Odhner (1910). The latter author pointed out the similarity of the genus *Microlistrum* Braun, with *Galactosomum* Looss, and expressed a supposition that these two genera may be identical. Meanwhile, these two genera must remain separated because of differences in the arrangement of the genital glands.

According to Jägerskjöld (1908), a fourth species may be added to this genus, *Monostomum semifuscum* Olsson, 1876. However, as the original description is insufficient and the types of this species are lost, a re-description of new material from the same host—*Sula basana*—is necessary to verify this point.

Tribe *CRYPTOCOTYLEA* n. tr.

Diagnosis: *Heterophyinae*, in which the vitellaria extend anteriorly up to the level of the genital pore or beyond it.

Type genus:—*Cryptocotyle* Lühe, 1899.

Although the earlier authors have distinguished the sub-families *Cryptocotylinae* (Lühe, 1899), *Tocotreminae* (Looss, 1899), *Apophallinae* (Ciurea, 1924), which partly correspond to the tribe *Cryptocotylea*, the latter could not hitherto be created as incorrect criteria have been used for classification.

The above sub-families were based on the details of the ventro-genital sac, while in fact characters of higher rank only must be used for making a sub-family. The creation of a sub-family for this group of *Heterophyidae* seems, however, to be previous. This step may be justified later, when many still undescribed forms will be revealed.

Genus *Cryptocotyle* Lühe, 1899.

The genus *Cryptocotyle*, 1899, was made almost at the same time as the genus *Tocotrema* Looss, 1899. Since the type species of both these genera are characterised by similarly constructed ventro-genital sacs, i.e., by similar characters used for generic identification, the name *Tocotrema* was suppressed in favour of *Cryptocotyle* by all later writers.

According to my experience, the structure of the ventro-genital sac may unite into one genus only species in which other essential characters are similar. In the species designated as types for *Cryptocotyle* and *Tocotrema* essential differences exist in the arrangement of the testes and in the shape of the body, both these characters being correlated. Thus, these two species cannot be retained in one genus but must be separated, i.e., both *Cryptocotyle* and *Tocotrema* should be considered valid.

The diagnosis of the genus *Cryptocotyle* may be modified as follows:—

Heterophyinae. Body almost round or pentagonal, praepharynx short; oesophagus well developed; the rudimentary ventral sucker is included in the ventro-genital sac; the testes lie side by side at the posterior margin of the body; the ovary in front of the

right-testis* ; the seminal receptacle in front of the testes on the middle line ; the seminal vesicle (?) ; the vitellaria extend anteriorly beyond the level of the genital pore ; the uterus coils between the levels of the ovary and ventro-genital sac ; the latter is situated near the middle of the body ; the excretory vesicle Y- or T-shaped, with short stem and branches. Adults parasitise mammals and birds.

Type species :—*C. concavum* (Creplin, 1825).

In addition to the type species two more species should be included in this genus—*C. quinqueangulare* (Skrjabin, 1923) and *C. cryptocotyloides* (Issaitschikoff, 1923). Both these species have been originally described under the generic name *Ciureana* Skrjabin, 1923. The above genus was thought to differ from *Cryptocotyle* only in having bean-shaped eggs, a feature more marked in *C. quinqueangulare* than in *C. cryptocotyloides*. This feature must, however, be considered as not more than specific and therefore I consider the creation of the genus *Ciureana* to be unjustified.

The existing three species of the genus *Cryptocotyle* may be determined according to the following key :

A. Testes lobed or serrated :

- (1) eggs oval.....*C. concavum* (Creplin, 1825)
- (2) eggs bean-shaped*C. cryptocotyloides*
(Issaitschikoff, 1923)

B. Testes not lobed*C. quinqueangulare*
(Skrjabin, 1923)

I had no representative of this genus in my collection and can therefore add no details concerning them.

(Genus *Tocotrema* (Looss, 1899).

Diagnosis : *Heterophyinae*.—The body elongated; oval or tongue-shaped ; the praepharynx and oesophagus vary in length ; the testes lie in the hind extremity of the body, obliquely to its axis ; the seminal receptacle in front of the right axis ; the ovary in front of the seminal receptacle ; the seminal vesicle long and coiled, passes into an elongated expulsor ; the vitellaria may reach

*Several authors described the ovary as situated in other places but these assertions are probably erroneous.

anteriorly up to the intestinal bifurcation; the uterine coils lie between the level of the ovary and the genital aperture; the ventro-genital sac is situated on the middle line of the body, contains a conical gonotyl and on the dorsal wall a depression which may be regarded as a rudiment of the ventral sucker (?); the excretory vesicle is approximately Y-shaped, but the stem is long and curved like an S, while the right branch is somewhat shorter than the left one. Adults parasitic in mammals and birds.

Type species:—*T. lingua* (Creplin, 1825).

Three species should be referred to the genus *Tocotrema*; *T. lingua* (Creplin, 1825), *T. jejunum* Nicoll, 1907, and *T. echinata* (Linstow, 1878), but I was not able to assure myself of the validity of the last two. I have not had the material for comparison but the specimens of *T. jejunum* kindly sent to me by Professor Ciurea may be equally diagnosed as *T. lingua*.

The validity of *T. echinatum* is problematical, as the original description is very inadequate. Lühe (1909) regarded it as belonging to the genus *Cryptocotyle*, but as its testes are arranged obliquely it cannot remain in that genus. Ciurea (1924) maintains that the specimens in America are distinct from the type and proposes for them a new name—*Cryptocotyle americanum*. As a justification for this emendation he records some insignificant differences from *T. lingua*, which may be due to the method of fixation, but are certainly not of specific importance. Further comparative study should clear up this confusion.

Genus *Rossicotrema* Skrjabin, 1919.

The genus *Rossicotrema* was created by Skrjabin with *R. donicum* Skrjabin, 1919, as type. Ransom (1920), without taking into account the work of Skrjabin, published his new genus *Cotylophallus* with two species—*C. similis* and *C. venustus*. As Ciurea pointed out, these two genera are identical and the name *Rossicotrema* should be retained as valid.

A study of the cotype specimens of *Apophallus brevis* Ransom, 1920, kindly sent me by Professor J. Ciurea, showed that this species must also be regarded as a member of the genus *Rossicotrema*. Thus, four species should be attributed to the above genus,

R. donicum Skrjabin, *R. simile* (Ransom), *R. venustum* (Ransom), and *R. breve* (Ransom). The detailed comparison of data in the literature shows, however, that the most essential differences between the species mentioned are only in the thickness and distension of the vitellaria follicles. This feature cannot, of course, be acknowledged as important enough to decide on the validity of species, and therefore I hold all the above-mentioned specific names as synonyms of *Rossicotrema donicum* Skrjabin, 1919.

The diagnosis of the genus *Rossicotrema* should be modified as follows :—

Heterophyinae. Body oval, narrower anteriorly than posteriorly ; the praepharynx short, oesophagus long ; ventral sucker included in the ventro-genital sac ; the testes lie at the posterior extremity of the body obliquely to its axis ; the seminal receptacle lies in front of the right testis ; the ovary in front of the seminal receptacle ; the seminal vesicle is composed of several (two or three) parts separated by constriction ; the vitellaria extend anteriorly almost up to the intestinal bifurcation ; the ventro-genital sac is situated on the middle line of the body and contains two small gonotyls arising at the sides of the genital aperture ; the excretory vesicle is Y-shaped with an S-like bent stem. The adults are parasitic in mammals and birds.

Type species :—*R. donicum* Skrjabin, 1919.

Genus *Apophallus* Lühe, 1909.

Three species of this genus have been hitherto described : *A. mühlingi* (Jägerskjöld 1899), *A. brevis* Ransom, 1920, and *A. major* Szidat, 1924. As previously shown, *A. brevis* is a synonym of *Rossicotrema donicum* Skrjabin. *A. major* is a synonym of *A. mühlingi*, the size is the only difference between them. This feature depends on the age of the worms and cannot be regarded as a character of specific importance. The findings of Ciurea (1924) confirm this supposition : this writer found specimens of *A. mühlingi* smaller than the type specimens and larger than the specimens described by Dr. Szidat. Professor Ciurea and Dr. Szidat kindly sent me their specimens and I was thus able to recognise the identity of these two species.

Among the specimens sent by Dr. Szidat there was one from a cat. It is the first record of a natural infection with *A. mühlingi* in a cat. This specimen is very small, and it is probable that cats are not suitable hosts for *A. mühlingi*.

The diagnosis of the genus *Apophallus* should be modified as follows :

Heterophyinae. Body very elongated ; praepharynx short, oesophagus long ; the globular ventral sucker is included in the ventro-genital sac ; the testes lie almost one behind the other in the posterior extremity of the body ; in front of them lie the seminal receptacle and the ovary ; the seminal vesicle is long and coiled and may be divided into several parts by constrictions ; the vitellaria extend from the posterior extremity of the body up to the level of the genital opening or still farther anteriorly ; the uterine coils lie between the levels of the ovary and genital pore ; the ventro-genital sac is situated on the middle line ; in addition to the ventral sucker it contains two tubercle-like gonotyls guarding the genital pore ; the excretory vesicle has a long S-like bent stem and short branches. Adults parasitise mammals and birds.

Type specimen :—*A. mühlingi* (Jägerskjöld, 1899).

SUB-FAMILY CENTROCESTINAE LOOSS, 1899.

Diagnosis : *Heterophyidae*.—The body rounded posteriorly, tapering anteriorly ; oral aperture surrounded by more or less conspicuous spines ; the oral sucker has, in some genera, an anterior lip-shaped appendage and a caudal conical appendage ; two testes situated posteriorly to other reproductive organs.

Type genus :—*Centrocestus* Looss, 1899.

This sub-family contains at present five genera which, similarly to *Heterophyinae*, might be divided into two tribes, i.e., according to the degree of development of the vitellaria. The number of genera is, however, too small to make the division necessary. The following is a key to the genera :—

A. The vitellaria do not extend anteriorly beyond the level of the ovary :

- (1) the caudal appendage of the oral sucker absent ...*Pygidiopsis* Looss, 1907
- (2) the caudal appendage of the oral sucker present ...*Parascacotyle* Stunkard and Haviland, 1924.

B. The vitellaria reach anteriorly up to the level of the genital aperture :

(1) the caudal appendage of the oral sucker absent ... *Centrocestus* Looss, 1899,
Stamnosoma Tanabe,
 1922

(2) the caudal appendage of the oral sucker present ... *Ascocotyle* Looss, 1899

It is seen that the genera *Centrocestus* and *Stamnosoma* are not separated in this key. In fact the differences between them are so slight that they may be regarded as identical. If one compares the discussion of Faust and Nishigori (1926) on the validity of the genus *Stamnosoma*, and the diagnosis of *Stamnosoma* cited by Stiles and Hassall (1926) with the original description of *Centrocestus*, the conclusion is reached that the differences between these two genera are not of generic rank. I leave, however, the genus *Stamnosoma* since I was not able to examine the types or original descriptions.

I had only the representatives of the genus *Pygidiopsis* and *Parascocotyle* at my disposal.

Genus *Pygidiopsis* Looss, 1907.

Diagnosis : *Centrocestinae*.—The pear-shaped body divided into an almost globular posterior part and a concave anterior part ; the oral sucker has no appendages ; praepharynx long, oesophagus short ; the ventral sucker is included in the genital sac ; the testes lie at the posterior margin of the body, side by side ; the seminal vesicle, in front of the testes on the middle line ; the ovary, ventrally to the seminal receptacle ; the seminal vesicle may be divided in parts by constrictions ; the vitellaria are situated at sides of the testes ; the uterus coils between the levels of the ovary and the genital sac ; the ventro-genital sac is situated on the middle line and contains a small gonotyl in its left anterior angle ; the excretory vesicle is T-shaped with the branch equal to the stem. The adults parasitise mammals and birds.

Type species :—*P. genata* Looss, 1907.

Pygidiopsis genata Looss, 1907.

(Fig. 22).

This species was found by Looss (1902) in the pelican in Egypt, by Ciurea (1924) in Rumania, in the dog by Faust in China and by Linton (1928) in the United States in *Butorides virescens* (see

Ascocotyle plana). I found this species among the Trematodes sent from the Berlin Museum (host, Persian wolf).

In Palestine this species is a rare parasite of dogs and cats. The second intermediate hosts in Palestine are *Tilapia galilea*, *T. nilotica*, *T. simonis* and *Barbus canis*.

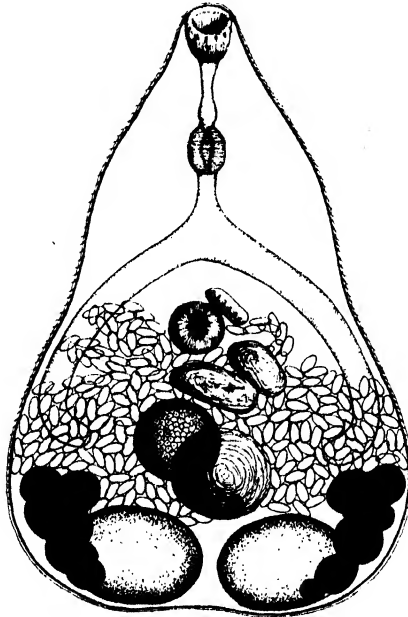


FIG. 22. *Pygidiopsis genata*, from the dog.

The body consists of an almost globular posterior part and a flattened anterior one. Normally the worm is bent ventrally with the anterior part concave; when flattened it is pear-shaped or triangular. The length of mature worms is 0.4–0.7 mm., the breadth 0.2–0.4 mm. Except for the hind portion the whole body is covered with thick scale-like spines. Round the oral aperture there is a row of spines, 16 in number, which are twice as long as those of the integument and are only seen on fresh material.

The oral sucker is oval or globular, 0.03–0.05 mm. wide and does not possess a funnel-like appendage. The praepharynx is 0.03–0.10 mm. long, and when contracted shows a bulb-like dilation posteriorly. The pharynx is 0.02–0.04 mm. long. The oesophagus

is short—0.03–0.06 mm. The caeca reach the level of the ovary and there turn somewhat dorsomedially.

The testes are round or transversely oval, 0.06–0.14 mm. in diameter. They lie side by side in the posterior extremity of the body. A large globular seminal receptacle, 0.07–0.14 mm. wide lies immediately in front of the testes on the middle line of the body near its dorsal surface. A globular ovary 0.04–0.08 mm. in diameter lies a little to the right and ventrally to the seminal receptacle. Two seminal receptacles of varying sizes, separated from each other by a constriction, which may disappear on distension by spermatozoa, are situated on the left side of the body, in front of the seminal receptacle, one behind the other. The second vesicle is connected by an ejaculatory duct with the terminal portion of the uterus.

The vitellaria are situated at the angles of the posterior extremity of the body and consist each of five to eight large pressed follicles arranged in single longitudinal rows. The uterus fills the free space between the testes and the ventro-genital sac and opens in the left angle of its anterior wall.

The ventro-genital sac is situated at the middle of the body and is occupied by a globular ventral sucker 0.04–0.06 mm. in diameter. In the left angle of the ventro-genital sac near the genital aperture there is a small oval gonotyl, the so-called 'lenticular-shaped body' 0.04–0.06 mm. in the long axis. In some cases the ventral sucker may partly protrude from the sac but the gonotyl is always covered by the dorsal border of the latter.

The eggs are oval, 0.018–0.022 mm. long and 0.009–0.012 mm. wide and are provided with a conspicuous filament on the posterior pole.

Genus *Parascocotyle* Stunkard, 1924.

Up to the present five distinct species of this genus have been described. They have all been attributed by the earlier authors to the genus *Ascocotyle* Looss. Stunkard and Haviland (1924) defined the generic peculiarities of *Ascocotyle* and showed that the type specimen of this genus *A. coleostoma* (Looss) differs so markedly from other allied species that it appeared necessary to separate it from them. They therefore created the genus *Parascocotyle* for the remaining species.

In the genus *Ascocotyle* there are two rows of circumoral spines and several coils of the uterus wind in front of the genital aperture, while in the genus *Parascocotyle* there is only one row of circumoral spines and the coils of the uterus confine themselves behind the genital aperture. Another essential difference between these two genera, which Stunkard and Haviland did not point out, is the position of the vitellaria, which in *Ascocotyle* extend in front of the ventral sucker, while in *Parascocotyle* they do not pass anteriorly beyond the level of the ovary.

It should, however, be pointed out that, while the distinction of the genus *Parascocotyle* by Stunkard and Haviland is fully justified, the creation of the species *Parascocotyle diminuta* by these authors appears to be unnecessary. I consider *P. diminuta* as a synonym of *P. minima* (Looss), for the differences on which this species was established can be attributed to age or fixation and are not of specific value.

Ciurea (1924) stated that in species encountered in America the ventral sucker is included in the genital sinus, while in others it lies on the ventral surface of the body, and on this basis all the species might be divided into two genera. This view is based on a misinterpretation of the position of the ventral sucker, which has been wrongly described by previous authors as lying above the ventral surface of the body. Probably in all species of *Parascocotyle* the ventral sucker is included in the genital sac, at least in the majority of them, as I have had occasion to observe.

Diagnosis: *Centrocestinae*.—Body pyriform; the dorsal wall of the oral sucker is provided with a contractile triangular lip-like appendage anteriorly and with a conical appendage posteriorly; the oral aperture is surrounded by a single row of conspicuous spines; praepharynx long, oesophagus short; the ventral sucker is included in the genital sac; the testes lie side by side at the posterior margin of the body; the seminal receptacle in front of them on the middle line, the ovary in front of the right testis; the seminal vesicle is divided in parts by constrictions; there is no marked expulsor; the vitellaria are situated at the sides of the testes; the uterus coils between the testes and the genital opening; the ventro-genital sac is situated on the middle line and contains besides the globular or oval ventral sucker, one or two tubercle-like gonotyls, the surface

of which may bear chitinized bars; the excretory bladder is Y- or T shaped. Adults parasitise mammals and birds.

Type species: *P. minuta* Looss, 1899.

The following is a key to all known species of the genus *Parascocotyle*, including a new species *P. ascolonga*. *P. pithecophagicola* (Faust, 1920), which is included in this key, is insufficiently described and requires further study for justifying its systematic position or even validity.

A The caeca reach only up to the level of the ventral sucker :

- (1) adequately described species *P. minuta* (Looss)
- (2) insufficiently described species *P. pithecophagicola* (Faust)

B. The caeca reach the ovary or more posteriorly :

- (1) the vitellaria compact :
 - (a) the appendix of the oral sucker reaches the pharynx *P. ascolonga* n. sp.
 - (b) the appendix of the oral sucker half as long as the praepharynx *P. italica* (Alessandrini)
- (2) vitellaria divided into follicles :
 - (a) the uterine coils entangled; one muscular papilla in front of the genital aperture *P. nana* (Ransom)
 - (b) the uterine coils have a transverse direction; there are two muscular papillae in front of the genital aperture *P. longa* (Ransom)

Parascocotyle longa (Ransom, 1920).

(Figs. 23 and 24).

This species which has hitherto been reported only from the Alaskan fox was frequently observed in Palestinian dogs and cats. I also found this species among other *Heterophyidae* in a bottle labelled '*Cotylogonimus persicus* from the Persian Wolf. (No. 3935).' Braun, who examined this material, evidently overlooked specimens of *P. longa*.

In dogs the worms occur mainly in the hindgut.

The secondary hosts in Palestine are *Mugil cephalus*, *M. capito*, *Lichia amia*, *Barbus canis*.

Parascocotyle longa is the largest species of the genus, the length reaching 0.5-1.2 mm., the width 0.3-0.4 mm. Normally the worms

are pear-shaped, elongated and concave ventrally. The body, except the posterior extremity, is covered with minute scale-like spines.

The oral sucker has the appearance of a beaker 0.05–0.06 mm. wide with a funnel-like appendage (but not an empty sac, as thought by some authors). The praepharynx is 0.14–0.37 mm. long, passes

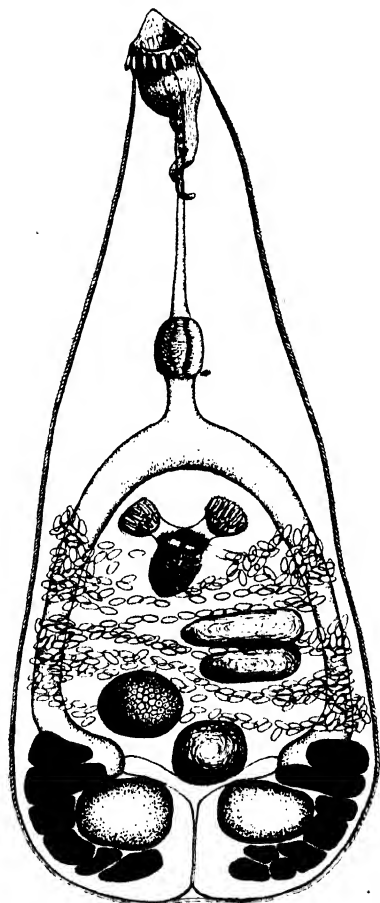


FIG. 23. *Parascocotyle longa*, from the dog.

ventrally to the caudal appendage of the oral sucker and reaches up to the end of the first third of the body, while the appendage extends for not more than two-thirds of this distance. The appendage

is generally bent like an **S** and is apparently non-contractile; however, its configuration may vary in different specimens (compare figs. 23 and 24).

The oral sucker is surrounded by a single row of 16 large spines, 18–20 μ long. The dorsal border of the oral sucker projects as a triangular lip which is not apparent on contraction. The pharynx is 0.05–0.07 mm. long. The oesophagus is short and is not seen in very contracted specimens. The caeca reach up to the testes; their ends bend slightly dorso-medially.

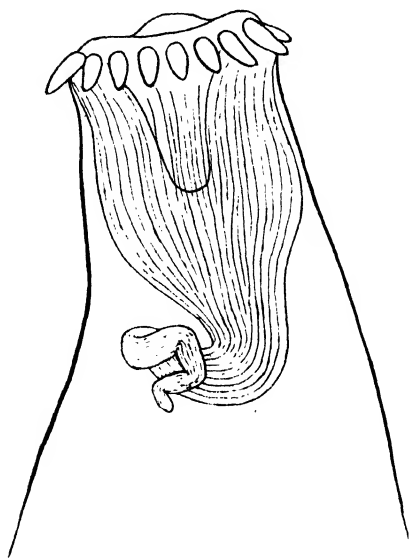


FIG. 24. The oral sucker of *Parascoctyle longa*, with a contracted lip and bent posterior appendage.

The testes, 0.06–0.12 mm. in diameter, are round or oval transversely. They lie side by side at the posterior extremity of the body and when large they are contiguous. The globular ovary is 0.04–0.08 mm. in diameter, and lies in front of the right testis and some distance from it.

The large globular seminal receptacle lies on the middle line of the body just in front of the testes. A small Mehlis' body is situated behind the seminal receptacle. The vitellaria are situated laterally to the testes. They consist of five to eight large globular follicles

arranged in single rows. The second follicle (counted backwards) is always very wide and extends internally beyond the others.

The vasa efferentia open into the large seminal vesicle, which is \supset -shaped or consists of two vesicles separated from each other by a constriction. The seminal vesicle lies in the left side of the body midway between the seminal receptacle and the ventro-genital sac. From the upper part of the seminal vesicle an ejaculatory duct arises which unites with the terminal portion of the uterus just before the genital opening.

The uterus fills the whole free space between the level of the ovary and the genital opening. It makes five to eight transverse coils which pass from side to side of the body, and open on the dorsal wall of the ventro-genital sac. The latter is situated in the middle of the body in the centre of a conspicuous elevation. The sac is filled by the rudimentary oblong ventral-sucker, 0.04–0.06 mm. in diameter, usually directed obliquely. In the anterior wall of the sac there is a transverse slit, in the middle of which the genital opening is situated. On each side of the genital opening there is a depression filled by a tubercle-like gonotyl 0.02–0.04 mm. wide, of irregular shape. The gonotyls bear on their surface nine to twelve minute chitinous bars.

The eggs are typical for the genus, i.e., they are small, 0.018 by 0.09 mm., almost transparent, with a thin shell and flat operculum.

Parascocotyle italica (Alessandrini, 1906).

(Fig. 25).

Four specimens of this species were found on one occasion in a dog in Jerusalem together with some specimens of *Parascocotyle ascòlonga* and numerous specimens of *Parascocotyle longa*.

The worms are pear-shaped, 0.7–0.8 mm. long and 0.2–0.3 mm. wide. Except the hindmost portion of the body the whole surface is covered with small scale-like spines.

The oral sucker 0.06 mm. in diameter is beaker-shaped; its dorsal wall bears a triangular retractile lip, which may disappear on contraction, and behind a funnel-like appendage ending at the level of the middle of the oesophagus. The praepharynx is 0.09–0.10 mm., pharynx 0.05 mm. and oesophagus 0.03–0.04 mm. long.

The caeca are two to three times as wide as the oesophagus and reach the level of the ovary where they bend slightly dorso-medially.

The testes are globular, 0.08 mm. wide, and lie side by side in the hindmost portion of the body. A large seminal receptacle lies in front of them in the middle line. The globular ovary 0.05–0.06 mm. in diameter lies on the right and a little in front of the seminal receptacle. The vitellaria are two solid masses of irregular outline lying along the lateral borders in the last quarter of the body.

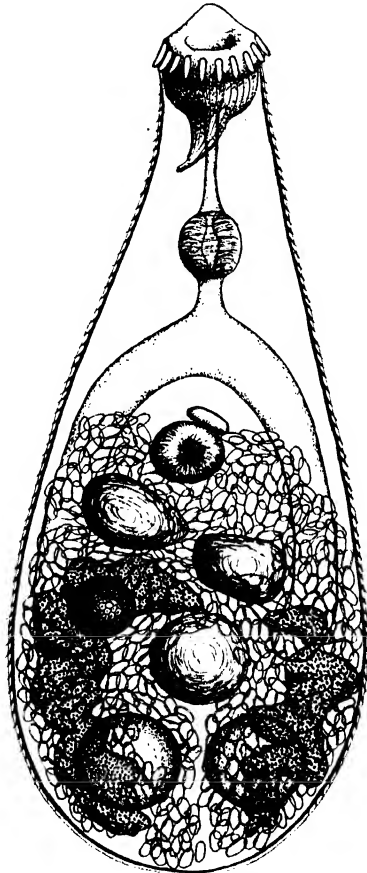


FIG. 25. *Parascocotyle italica*, from the dog.

The coils of the uterus fill the whole free space behind the genital aperture. The vasa efferentia open into one of two large transversely oval united seminal vesicles situated between the seminal receptacle and the genital aperture.

The latter opens on the dorsal wall of the ventro-genital sac, between the base of the globular ventral sucker (0.05–0.06 mm. in diameter) and a small oval gonotyl. The oval eggs (0.019 mm. long, 0.009–0.010 mm. wide), are narrowed at the anterior pole, and have a thin shell.

This species closely resembles *P. nana* (Ransom). The only essential difference between these two species lies in the arrangement of the vitellaria. In *P. italica* the latter form solid masses while in *P. nana* they are divided into follicles. I ascertained this difference on comparing my specimens with cotypes of *P. nana*, kindly sent me by Professor M. C. Hall.

Parascocotyle ascolonga n. sp.

(Figs. 26–28).

I found this species in dogs and cats, usually in small numbers. Experimentally it was obtained by feeding dogs with *Tilapia simonis*, and *T. galilea*.

The body is 0.5–0.7 mm. long, is spindle- or pear-shaped with the maximum breadth in the posterior extremity (0.1–0.3 mm. broad). Except the hindmost part, the whole body is covered with small scale-like spines.

The oral sucker has the shape of a beaker 0.04–0.07 mm. wide, bearing in front a retractile triangular lip and provided beneath with a long funnel-shaped solid appendix which reaches the anterior margin of the pharynx, and in contracted specimens may extend even beyond this. The oral aperture is surrounded by a single row of 16 large spines 0.018–0.022 mm. long. The praepharynx is 0.04–0.15 mm., pharynx 0.02–0.04 mm., oesophagus 0.009–0.018 mm. long.

The intestinal caeca are of equal width and reach the level of the seminal receptacle, where they turn somewhat dorso-ventrally.

The testes, 0.04–0.10 mm. in diameter are usually globular and lie side by side in the hind extremity of the body; occasionally they are contiguous. A large seminal receptacle, the size of which depends on the amount of distension with spermatozoa lies in front of the testes on the middle line. The globular or transversely oval ovary, 0.02–0.06 mm. long and 0.06–0.07 mm. wide, lies a little in front and to the right of the seminal receptacle. The vitellaria appear as two

elongated masses lying along the border of the hind extremity of the body; anteriorly they reach the level of the ovary.

The vasa efferentia open into one of the two voluminous seminal vesicles which lie midway between the seminal receptacle and the ventro-genital sac. The ejaculatory duct, which arises from the second seminal vesicle, opens into the terminal portion of the uterus just before the genital aperture.

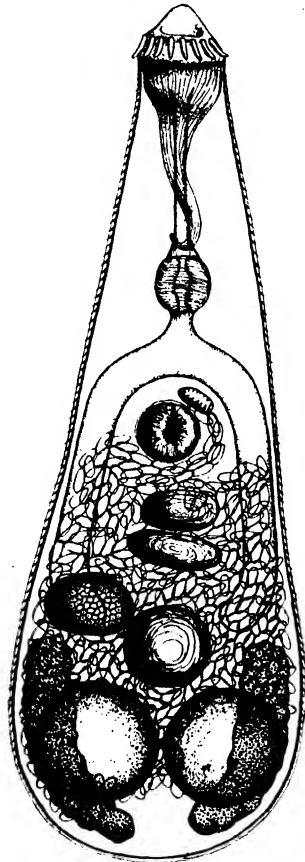


FIG. 26. *Parascocotyle ascolonga*, from the dog.

The tangled coils of the uterus fill the whole free space between the genital aperture and the testes and often extend behind the latter. The genital sac lies almost in the middle of the body. It is filled

by a round ventral sucker 0.04–0.05 mm. in diameter. On the left side of the anterior wall of the ventro-genital sac there is a slit at the bottom of which is situated a small tubercle bearing the genital aperture. Between it and the aperture of the slit lies a small oval gonotyl 0.02–0.03 mm. in long axis. This slit is contractile and when contracted, the gonotyl may be extruded.

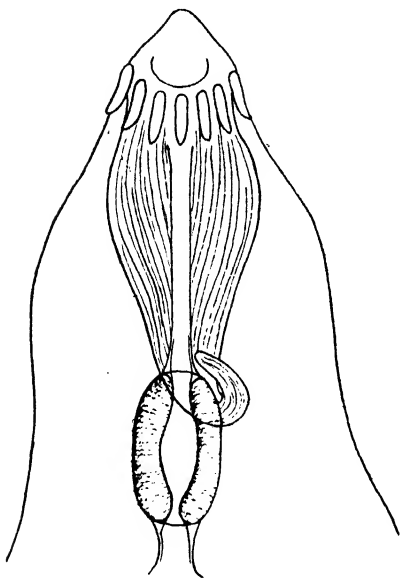


FIG. 27. The contracted anterior extremity of *Parascocotyle ascolonga*.

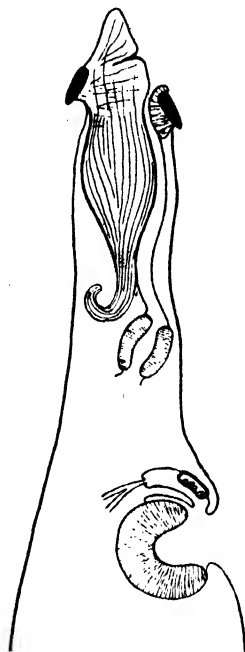


FIG. 28. Longitudinal section through the anterior portion of *Parascocotyle ascolonga* (semi-diagrammatic).

The eggs are 0.018 mm. long, 0.009 mm. wide, with thin shells, somewhat narrowed anteriorly and with distinctly visible opercula.

This species resembles *P. nana* (Ransom) on the one side and *P. italica* (Aless.) on the other. It differs from the first in the length of the appendix of the oral sucker, which always reaches up to the pharynx, independently of the amount of contraction or distension, but not up to the middle of the praepharynx, and in the arrangement of the vitellaria which are compact masses, and not divided into follicles.

From *P. italica* (Aless.) it differs only in the character of the appendix of the oral sucker. I separated the latter two species only because I found no intermediate conditions in the specimens examined. In the four specimens of *P. italica* the appendix of the oral sucker was uniformly short while in all the specimens of *P. ascolonga* examined this appendix was found to reach the pharynx.

I assume that the funnel-like appendix of the oral sucker in the representative of the genus *Parascocotyle* may change its position, but does not contract to any extent, and therefore its relative length may serve as a specific character.

SUB-FAMILY CERCARIOIDINAE n. subf.

Diagnosis : *Heterophyidae* with flattened and markedly dilated anterior part of the body, without circumoral spines, with two testes situated behind the ovary.

Type genus : *Cercarioides* n. gen.

Besides the type genus this family also contains the genus *Scaphanocephalus* Jägerskjöld, 1903. The differences between these two genera are shown in the following table :—

	<i>Cercarioides</i>	<i>Scaphanocephalus</i>
Shape of the dilation	Inverted heart	Irregular with folded edges
Oral sucker	Large	Small
Position of the testes	Removed from the posterior extremity of the body	In the posterior extremity of the body
Position and extent of the vitellaria	Posteriorly to the anterior testis	Up to the intestinal bifurcation

Genus *Cercarioides* n. gen.

Diagnosis : *Cercarioidinae*.—Body elongated, divided into two distinctly separated parts : the anterior one flat heart-shaped and the posterior one spindle-shaped, oval in cross-section. Praepharynx

and oesophagus very short. The testes lie in the middle of the posterior part of the body one behind the other. In front of them in the order named lie the ovary and the seminal vesicle. The vitellaria are scattered in the posterior part of the body, posteriorly to the ovary. The uterus fills the whole open space in the posterior part of the body. The ventro-genital sac lies in the middle of the constriction dividing the body.

Type species :—*C. aharonii* n. sp.

Cercarioides aharonii n. sp.

(Fig. 29).

I found only one specimen of this species in *Puffinus kuhli*, caught near Suez. Its name is given in honour of Mr. J. Aharoni, the indefatigable ecologist of Palestine to whom I am indebted for the determination of the definite and intermediate hosts cited in this paper.

The length of the whole body is 3.4 mm. The first third of the body is flattened and is heart-shaped with the base posterior. It is connected with the posterior spindle-shaped part of the body by a narrow constriction. The relation between the lengths of these two parts is 1 : 2. The anterior part is covered with long and thin spines, while the first half of the posterior part with much shorter but thicker spines. The end of the body is free from spines.

The large oral sucker 0.38 mm. in diameter is situated ventrally on the anterior extremity. It is followed by the pharynx 0.14 mm. in diameter, which passes immediately into the intestine. The caeca first pass obliquely forward before reaching half-way to the edges of the body, turn towards the constriction and then become somewhat narrowed. They pass along the sides of the body up to 0.43 mm. from its posterior extremity.

The ventral sucker is probably included in a small ventro-genital sac, which is 0.05 mm. in diameter and lies on the middle of the constriction between the two parts of the body.

The slightly lobed testes lie in the middle third of the body. The posterior testis, 0.38 mm. in diameter lies near the right caecum. The ovary is globular, 0.18 mm. in diameter and lies near the right caecum, midway between the anterior testis and the ventro-genital sac. Immediately behind it lies the Mehlis' body.

The vitellaria consist of many large follicles of irregular shape

scattered throughout the whole posterior part of the body, behind the ovary.

The vasa efferentia flow into a wide vas deferens which opens into an oval first seminal vesicle measuring 0.15 by 0.09 mm. The latter opens into the second small seminal vesicle 0.04 mm. wide. Both vesicles lie between the ovary and the ventro-genital sac.

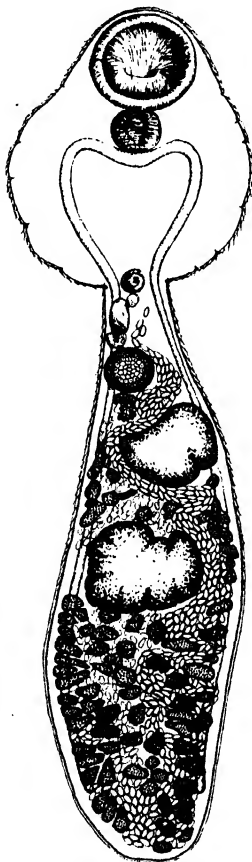


FIG. 29. *Cercarioides abaronii*, from *Puffinus kuhli*.

The coiled uterus fills the whole free space of the posterior part of the body. The genital aperture was not well seen in the only mounted specimen, but seems to open into the ventro-genital sac. The course of the excretory bladder was not determined. The eggs are oval, 0.037 mm. long and 0.022 mm. wide.

Sub-family *HAPLORCHINAE* (Looss, 1899) Poche, 1926.

This sub-family was created by Looss, under the incorrect name *Haplorchiinae* to contain the genera *Haplorchis* and *Galactosomum*. In the present paper the latter genus is assigned to the *Heterophyinae* and the genus *Monorchitrema* is included into the *Haplorchinae*. This sub-family is very near to the *Heterophyinae* and might be united with it but for the different number of testes.

Diagnosis: *Heterophyidae* with flattened but not dilated anterior part of the body; circumoral spines absent; one testis; the ovary and seminal receptacle situated in front of the testis.

Type genus:—*Haplorchis* Looss, 1899.

Genus *Haplorchis* Looss, 1899.

I had no representatives of the genus *Haplorchis* at my disposal and therefore cannot have a clear view of its peculiarities, for it is highly probable that in 1899 Looss did not use the most essential characters for classification. I include this genus as the published figures of its members bear a close resemblance to the genus *Monorchitrema*, which belongs to the *Heterophyidae*. It is, however, highly possible that after the revision of the type-specimens these genera will prove identical and the genus *Monorchitrema* will fall into synonymy.

Two species of the genus *Haplorchis* are known: *H. cahirinus* (Looss, 1896) and *H. pumilio* (Looss, 1896). It is noteworthy that the first is the only species of *Heterophyidae* found in the adult stage as a parasite of fish. This circumstance leads to the supposition that *H. cahirinus* may belong to quite another family.

Genus *Monorchitrema* Nishigori, 1924.

Diagnosis: *Haplorchinae*.—Body oval or elongated; praepharynx absent, oesophagus long; ventral sucker absent; the testes lie at the posterior extremity of the body, the ovary in front of it; seminal receptacle near the right border of the body; the seminal vesicle consists of several parts; an expulsor may be present; the vitellaria lie in the posterior part of the body behind the level of the ovary; the uterus coils at the sides of the testis and in front of it; the genital sac is situated on the right side of the middle line and

contains a large gonotyl which bears on its surface chitinous rodlets or other armature ; the excretory vesicle is Y-shaped. The adults parasitise mammals and birds.

Type species :—*M. taihokui* Nishigori, 1924.

Monorchitrema taihokui Nishigori, 1924.

(Fig. 19).

This species was discovered in Formosa as a parasite of a bird *Nycticorax nycticorax*. Experimentally it has been obtained by Faust and Nishigori in man, dog, cat and in small laboratory animals. The first intermediate host in Formosa proved to be a fresh-water snail *Melania reiniana* var. *hidachiensis*, the second—12 species of fish of the families *Cyprinidae*, *Siluridae* and *Colitidae*.

In Palestine this species is a rather rare parasite of dogs and cats and is seldom present in large numbers. I found it on one occasion together with *Monorchitrema taihui* and *Dexiogonimus ciureanus* in a *Larus* sp. caught near Lake Tiberias. I have obtained it experimentally in dogs and cats by feeding them with *Barbus canus*, *Barbus longiceps*, *Tilapia simonis*, *T. galilea*, *T. nilotica*, *Mugil capito*.

It is probable that this species is a synonym of *Haplorchis pumilio* (Looss) but I have not had the possibility of determining this point.

Although Faust and Nishigori (1926) gave a detailed description of this worm, I give it below again, for some details require further elucidation.

The worms are elongated and have rounded extremities. The posterior part of the body is round in cross section, the anterior part is somewhat flattened. The length of the body is 0.4–0.7 mm., the maximum breadth 0.1–0.3 mm. The whole surface of the body is covered with small scale-like spines. The oral sucker is 0.04–0.05 mm. in diameter ; the praepharynx is short, almost disappears on contraction and when distended reaches 0.03 mm. in length ; the pharynx is 0.02–0.04 mm., the oesophagus 0.08–0.14 mm. long ; the intestinal bifurcation situated between the first and middle thirds of the body ; the caeca are three to four times as thick as the oesophagus, they narrow somewhat towards their ends and reach the boundary between the middle and the last third of the body.

The single large globular or long-oval testis is 0.07–0.12 by 0.05–0.10 mm. in diameter and lies in the hind part of the body ; just in front of it lies the globular ovary 0.03–0.06 mm. in diameter. To the right of the ovary lies the seminal receptacle, which varies in size. To the left and somewhat in front of the seminal receptacle lie two seminal vesicles separated from each other by a short neck. The first is fairly small, the second large. The large vesicle is connected with a small expulsor and the latter gives rise to a short ejaculatory duct which unite with the end portion of the uterus.

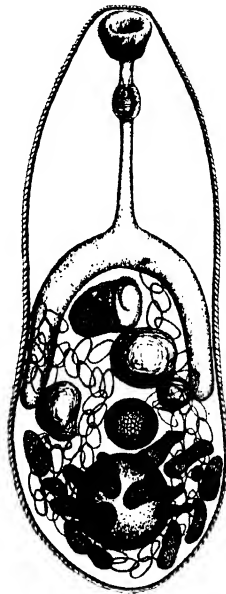


FIG. 19. *Monorchitrema taibokui*, from the dog.

The vitellaria are scattered under the dorsal surface of the body and consist of two united groups of large elongated follicles, 16 to 20 in number.

The uterus passes from the ovary to the left, bends round the testis, turns forward and after a sinuous course reaches the genital aperture, which opens in the median wall of the genital sac. The latter is situated dextrally to the middle of the body. It is occupied by a semi-circular gonotyl, which protrudes partly from the opening of the sac. This protruding part of the gonotyl forms the true

posterior and lateral border of the opening of the genital sac and bears on its surface a row of about 40 very minute spines. At the bottom of the sac there is a depression paved with large cells, clearly visible in total preparations as a darkly stained spot, which is apparently a rudiment of the ventral sucker.

The eggs are 0.028–0.032 mm. long and 0.014–0.018 mm. wide, they often possess a minute filament on the posterior pole.

Monorchitrema taihui Nishigori, 1924.

(Figs. 20 and 21).

This species was found in Formosa together with the one described above by feeding man and experimental animals with seven species of fish belonging to the families *Cyprinidae*, *Siluridae* and *Colitidae*. The first intermediate host of this species in Formosa is a snail *Melania oblique-granosa* (Smith).

In Palestine, mainly in the vicinity of the Lake Tiberias this species is not uncommon in dogs and cats. I found a few species on one occasion in *Larus* sp. near Lake Tiberias. The worms were smaller than those from carnivora.

Experimentally I obtained it after feeding dogs with fish: *Varicorhinus* sp., *Tilapia simonis*, *Barbus canis* and *Gambusia affinis*. In the latter species the metacercariae were found in the muscles, under the skin, in the fins and tail, while in others only in muscle.

This species is much larger than the previous one. They both frequently occur together but they can easily be distinguished by their sizes.

The length of the body is 0.5–1.2 mm., the maximum breadth 0.24–0.4 mm. The normally distended specimens are elongated in shape, with the anterior part flattened and the posterior one oval in cross section. The contracted specimens are oval in shape. Almost the whole body is covered with small spines which are longest on the first third of the body, becoming smaller towards the posterior extremity.

The oral sucker is 0.05–0.07 mm. in diameter. Praepharynx is seen only in very distended specimens in which it does not exceed 0.018 mm.; pharynx 0.02–0.05 mm. in length. The long oesophagus, 0.11–0.28 mm., is somewhat dilated posteriorly and reaches the

boundary between the first and the middle third of the body. The caeca are three to four times as wide as the oesophagus and reach the posterior extremity of the body.

There is only one very large testis, 0.12–0.21 mm. in diameter, filling, in some specimens, the whole space of the posterior quarter of the body. It is round or oval with the long axis longitudinal.

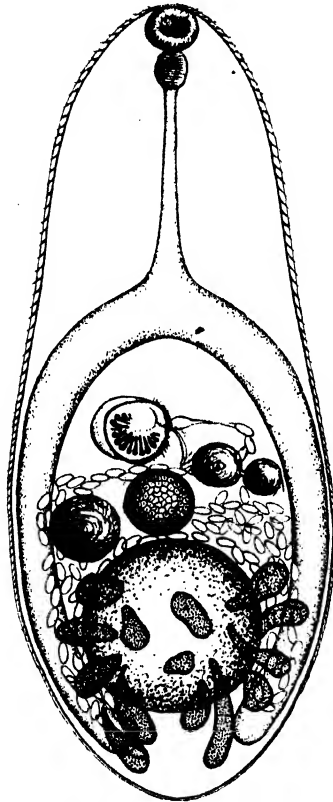


FIG. 20. *Monorchitrema taibui*, from the cat.

In front of the testis lies a globular ovary 0.05–0.11 mm. in diameter. To the right of the ovary lies the seminal receptacle which may be smaller or larger than the ovary.

The vasa efferentia open into the first small seminal vesicle which is separated by a constriction from second and larger one. From the

latter a short ejaculatory duct emerges and opens into the uterus some distance before the genital aperture.

The uterus proceeds from the ovary to the left, bends along the border of the testis, proceeds to the end of the left caecum, turns back, bends round the testis up to the end of the right caecum turns back, and finally proceeds to the genital aperture. Its terminal portion may be distended, forming a small egg-receptacle.

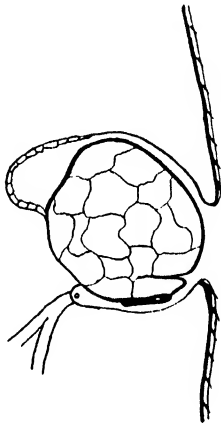


FIG. 21. Longitudinal section through the ventro-genital sac of *Monorchitrema taihii*.

The genital sac is situated to the left of the middle of the body and is ring-shaped (0.08–0.12 mm. diameter). It is occupied by a large gonotyl, which, owing to a slit in its median border, is almost semi-circular. The surface of the gonotyl is peculiarly ornamented by 14 to 18 chitinous bars 0.014–0.028 mm. long, arranged like a fan. On the hind median angle of the gonotyl there are again four to six conspicuous spines directed backwards. Dorsally to the gonotyl, in the bottom of the genital sac there is a depression paved with a layer of flat cells, which is apparently the rudiment of the ventral sucker.

The genital aperture opens on the median wall of the genital sac opposite to the slit of the gonotyl.

The eggs are oval, 0.025–0.028 mm. long, 0.012–0.015 mm. wide.

SUB-FAMILY ADLERIINAE NOV. SUBF.

Diagnosis : Very small *Heterophyidae* with oval or spindle-shaped body round throughout its whole length, without circumoral spines, with a single testis situated anteriorly to the ovary and the seminal receptacle.

Type genus : —*Adleria* n. gen.

Genus *Adleria* n. gen.

This generic name is dedicated to Dr. S. Adler through whose efforts the Helminthological Laboratory was established in the Hebrew University.

The diagnosis of this genus coincides with the diagnosis of the sub-family. As seen from the latter the anatomical structure of *Adleria* is an aberrant one when compared with other genera of *Heterophyidae*. I placed this genus among *Heterophyidae* because of the presence of a seminal receptacle, a seminal vesicle and a gonotyl.

Adleria minutissima n. sp.

(Figs. 30-33).

This very interesting trematode is found rather rarely in Palestinian dogs and cats, usually in small numbers. I obtained large quantities of these worms by feeding dogs with fishes : *Discognathus* sp., *Varicorhinus*, sp. *Barbus canis*, *Mugil cephalus*, *M. capito*.

The parasites are distributed throughout the whole length of the intestine of its host, but are most numerous in the first parts. They penetrate deeply into the villi and can be washed out only after their death. They remain alive for some fifteen minutes in scrapings of the intestinal mucosa where they are motile and change their shape as shown in fig. 33. They live only for one to two minutes in water.

These trematodes are very small, their length being 0.27-0.47 mm. their breadth 0.09-0.15 mm. The body is pear-shaped or spindle-shaped, round in cross section throughout its whole length. It is covered with thick spines which are absent only round the oral, genital and excretory apertures.

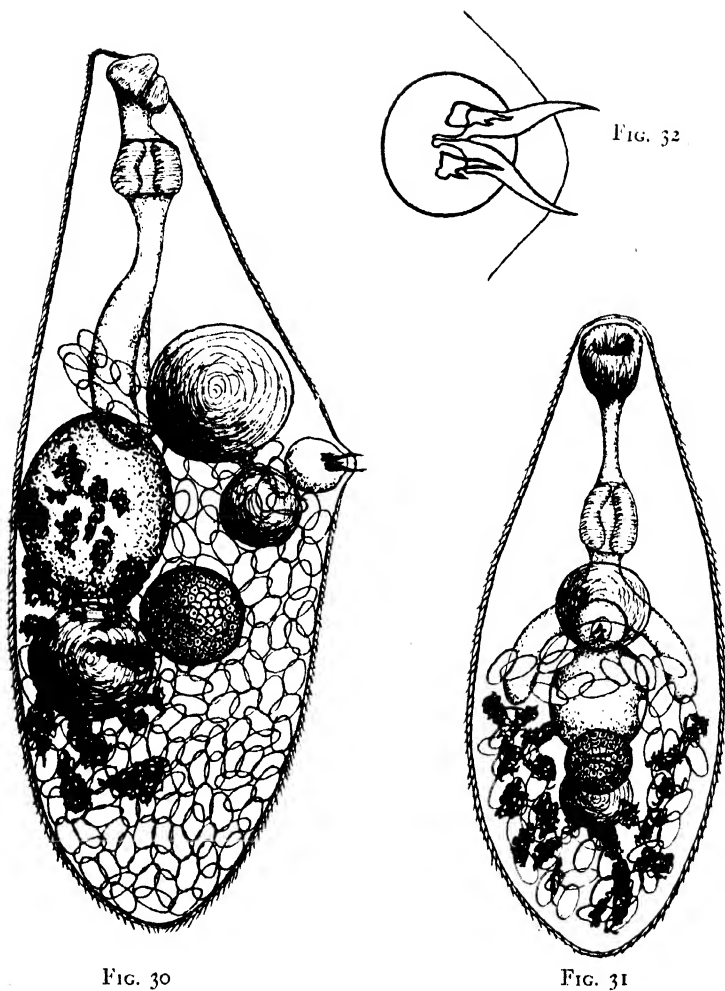


FIG. 30

FIG. 31

- FIG. 30. *Adleria minutissima*, lateral view of an adult specimen with contracted oesophagus.
 FIG. 31. *Adleria minutissima*, frontal view of a young specimen with distended oesophagus.
 FIG. 32. *Adleria minutissima*, gonotyl as seen from the side.

The oral sucker, 0.025–0.034 mm. wide, lies semi-ventrally. It is followed by the very contractile praepharynx, 0.015–0.050 mm. in length, which on contraction is three times shorter than when extended. During contraction it draws the caeca upwards. The pharynx is 0.025–0.031 mm., and the oesophagus 0.06–0.18 mm. long. The caeca are short ending in front of the middle of the body when the praepharynx is contracted, and behind it when it is

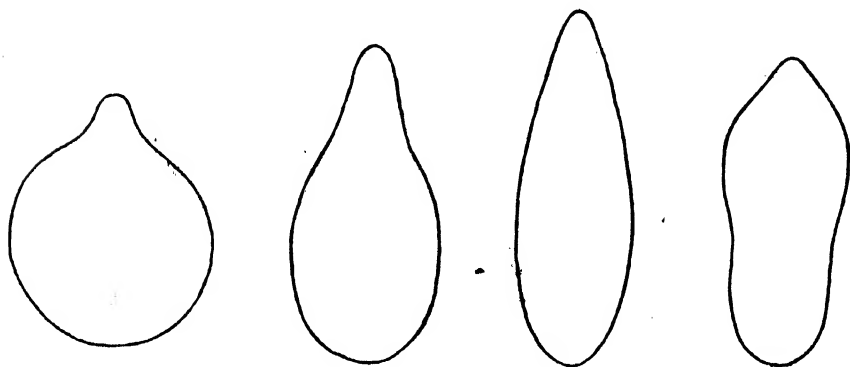


FIG. 33. Changes in shape of *Adleria minutissima* during the movement.

extended. There is only one large oval testis 0.04–0.10 mm. in length, with the long axis longitudinal. It lies just behind the middle of the body under its dorsal surface. Behind it lies a globular seminal receptacle 0.043–0.084 mm. in diameter. Ventrally to the testes and seminal receptacle lies a globular ovary 0.018–0.035 mm. in diameter. The latter is apparently reduced in adult specimens. In front of the testis, behind the intestinal bifurcation and the ventral surface of the body, lies a large globular first seminal vesicle 0.056–0.068 mm. in diameter which is connected with a second much smaller vesicle lying ventrally or posteriorly to it. The latter is not always present. The follicles of the vitellaria are scattered under the dorsal surface of the posterior half of the body.

The genital aperture opens into a genital sac 0.021–0.028 mm. in diameter. The latter is filled by a globular gonotyl which bears four spines—two large ones 0.015–0.018 mm. in length and two half that size. These spines project above the surface of the body.

The uterine coils fill the whole free space behind the genital aperture and often make the investigation of the internal anatomy of the mounted preparations very difficult.

The eggs are oval, 0.024 mm. in length and 0.012-0.014 mm. in width, and possess thick shells. They are probably seldom passed because they were very rare in the stools of dogs even in very heavy experimental infections.

V. REFERENCE LIST OF THE BIBLIOGRAPHY OF THE FAMILY HETEROPHYIDAE, INCLUDING ALL SPECIFIC, GENERIC, etc., NAMES, ALPHABETICALLY ARRANGED

Text-books and other compilations are omitted, unless they contain original descriptions or observations. When the original was not available the quotations of other authors have been cited. In preparing the following list, the Index Catalogue of Stiles and Hassall (1908) was largely used.

ABBREVIATIONS:

- Faust & N. = Faust and Nishigori.
Pres. = Described in the present paper.
Stiles & H. = Stiles and Hassall.
Stunkard & H. = Stunkard and Haviland.

The figures before the colon indicate the year of the cited paper, those after it the page; * indicates authors who have cleared up the synonymy; 'Pres.' indicates the present paper. The hosts are referred only to those authors who first recorded them.

Valid name	Described as	Author	Host
ADLERIA	ADLERIA	Pres.	
<i>Adleria minutissima</i>	<i>Adleria minutissima</i>	Pres.	<i>Felis catus dom.</i> , <i>Canis familiaris</i> ; metacercariae in <i>Barbus canis</i> , <i>Varicorbinus</i> sp., <i>Discognathus</i> sp., <i>Mugil cephalus</i> and <i>M. capito</i> .
ADLERIINAE	ADLERIINAE	Pres.	

Valid name	Described as	Author	Host
Apophallus	Apophallus	Lühe, 1909: 62 Odhner, 1914: 224 Skrjabin, 1919: 13 Ciurea, 1924: 18 Ransom, 1920: 529, 551-552 Nicoll, 1924: 168 Stunkard & H., 1924: 6 Faust & N., 1926: 91 Stiles & H., 1926: 91 Pres.	
<i>Apophallus müblingi</i>	<i>Apophallus müblingi</i>	Lühe, 1909: 62, fig. 53 Odhner, 1914: 224 Ransom, 1920: 552, 554, fig. 20 Kotlan, 1922 Nicoll, 1923a: 168, 191 Ciurea, 1924: 2, 4, 5, 10-12, 18, figs. 2, 7, 8 Stunkard & H., 1924: 2 Szidat, 1924: 2, 3, 4 Ruszkowski, 1925: 175 Poche, 1926: 148 Pres.	<i>Phalacrocorax carbo</i> , <i>Himantopus himantopus</i> , <i>Coccyzus erythrophthalmus</i> <i>Larus argentatus californicus</i> , <i>Pelecanus onocrotalus</i> , <i>Canis familiaris</i> (exp.); metacercariae in <i>Blicca björerna</i> <i>Felis catus</i> dom.
	<i>Apophallus major</i>	Szidat, 1924: 2-4, figs. 2a-3 *Pres.	<i>Larus fuscus</i>
	<i>Distoma lingua</i>	Mühling, 1898a: 21-22 Mühling, 1898b: 29, 94-96, fig. 16 *Jägerskjöld, 1899: 5-7 Szidat, 1924: 2	<i>Larus ridibundus</i>
	<i>Distoma müblingi</i>	Jägerskjöld, 1899: 7 *Lühe, 1909: 62	
	<i>Metorchis oesophagolongus</i>	Katsurada, 1914: 304-310, figs. 1-6, 10-11 Ciurea, 1924: 12	<i>Felis catus</i> dom.; metacercariae in <i>Acerina cernua</i> , <i>Abramis brama</i> , <i>Leuciscus rutilus</i> , <i>Idus idus</i> , <i>Blicca björerna</i>
	<i>Tocotrema müblingi</i>	Looss, 1899: 585	
Ascocotyle	Ascocotyle (s.l.)	Looss, 1899: 584-585, 586, 611 Braun, 1902: 30 Looss, 1902b: 441, 824, 832, 833 Pratt, 1902: 888, 894 Jägerskjöld, 1903: 14 Nicoll, 1907: 521 Odhner, 1914: 224 Skrjabin, 1919: 13 Ransom, 1920: 529, 561-562 Nicoll, 1923a: 168 Nicoll, 1923b: 239 Ciurea, 1924: 17 Dollfus, 1925: 192 Faust & N., 1926: 91	
	Ascocotyle (s. str.)	*Stunkard & H., 1924: 2-3, 6-7 Pres.	

Valid name	Described as	Author	Host
<i>Ascocotyle agrese</i>	<i>Ascocotyle agrese</i>	Travassos, 1916 : 1-2 Ransom, 1920 : 262, 264 Ciurea, 1924 : 17 Viana, 1924 : 97, 157 Stunkard & H., 1924 : 3	<i>Butorides striata</i>
<i>Ascocotyle coleostoma</i>	<i>Ascocotyle coleostoma</i>	Looss, 1899 : 582, 585, 699 Ransom, 1920 : 562-563, fig. 31 Ciurea, 1924 : 14, 17 Stunkard & H., 1924 : 3 Poche, 1926 : 148 Pres.	
	<i>Anoiktostoma coleostoma</i>	Stossich, 1899b : 15	
	<i>Distomum coleostomum</i>	Looss, 1896b : 101-106, 154, figs. 66-68 Braun, 1901a : 34	<i>Pelicanus oncorhynchus</i>
	<i>Distomum colostomum</i>	Vaullegeard, 1901 : 143 *Stiles & H., 1908 : 176	
CENTROCESTINAE	CENTROCESTINAE	Looss, 1899 : 586 Pratt, 1902 : 886, 894 Jägerskjöld, 1903 : 14 Ciurea, 1924 : 17 Stunkard & H., 1924 : 6 Viana, 1924 : 157 Dollfus, 1925 : 192 Stiles & H., 1926 : 78, 91 Pres.	
	PHAGICOLINAE	Faust, 1920 : 631 Poche, 1926 : 153 *Pres.	
CENTROCESTUS	CENTROCESTUS	Looss, 1899 : 584, 586 Braun, 1902 : 30 Pratt, 1902 : 888, 894 Jägerskjöld, 1903 : 14 Nicoll, 1907 : 521 Leiper, 1913a : 177 Odhner, 1914 : 244 Skrjabin, 1919 : 13 Ransom, 1920 : 529, 559 Nicoll, 1923b : 240 Ciurea, 1924 : 17 Stunkard & H., 1924 : 6 Faust & N., 1926 : 91 Stiles & H., 1926 : 91 Pres.	
<i>Centrocestus cuspidatus</i>	<i>Centrocestus cuspidatus</i>	Looss, 1899 : 584 Ransom, 1920 : 560-561, fig. 27 Nicoll, 1923b : 240 Ciurea, 1924 : 13-17 Faust & N., 1926 : 92, 121-122 Pres.	
	<i>Anoiktostoma cuspidatum</i>	Stossich, 1899b : 15 *Looss, 1899 : 582	

Valid name	Described as	Author	Host
<i>Cryptocotyle concavum</i>	<i>Distoma concavum</i>	Creplin, 1825 : 45-47, 83; figs. 7, 8 Creplin, 1837 : 310, 314, 318 Dujardin, 1845 : 448 Creplin, 1846 : 138, 141, 145, 146 Diesing, 1850 : 340-341 Cobbold, 1860 : 11 Stossich, 1892a : 158 Kowalewski, 1895 : 2 (35c) Braun, 1893a : 874-879 Stossich, 1898a : 10 Stossich, 1898b : 42 Mühling, 1898b : 4, 19-24, 27, 80-83, figs. 6, 20, 26 *Luhe, 1899 : 539 Jägerskjöld, 1899 : 9, 10, 12, 14, 16 Jacoby, 1899 : 22-23 Looss, 1899 : 586 Looss, 1900 : 607 Braun, 1900 : 6 Braun, 1901c : 564	<i>Gavia stellata</i> <i>Colymbus cristatus</i> , <i>Alca torda</i> , <i>Anas bornschubi</i> , <i>Glaucion</i> <i>clangula</i> , <i>Clangula hyemalis</i> , <i>Fuligula marila</i> , <i>Melanitta</i> <i>fusca</i> , <i>Mergus serrator</i> , <i>Merganser merganser</i> <i>Colymbus nigricollis</i>
	<i>Tocotrema concavum</i>	Looss, 1899 : 586 Kowalewski, 1902 : 26 Jägerskjöld, 1903 : 3, 4, 5, 11, 13	<i>Phalacrocorax aristotelis</i>
<i>Cryptocotyle cryptocotyloides</i>	<i>Cryptocotyle cryptocotyloides</i>	Pres.	
	<i>Ciureana cryptocotyloides</i>	Issaitschikoff, 1923 : 1-4, figs. 1-3 *Pres.	<i>Colymbus arcticus</i>
<i>Cryptocotyle quinqueangulare</i>	<i>Cryptocotyle quinqueangulare</i>	Pres.	
	<i>Ciureana quinqueangulare</i>	Skrjabin, 1923 : 4-6, fig. 1 Issaitschikoff, 1923 : 3, 4, fig. 4 *Pres.	<i>Felis catus dom.</i>
CRYPTOCOTYLEA	CRYPTOCOTYLEA	Pres.	
	<i>Apoballinae</i>	Ciurea, 1924 : 17 Stunkard & H., 1924 : 6 Stiles & H., 1926 : 91 *Pres.	
	CRYPTOCOTYLINAE	Lühe, 1909 : 86 Ciurea, 1924 : 18 Stunkard & H., 1924 : 18 Stiles & H., 1926 : 81, 91 *Pres.	
	TUCOTREMINAE	Jägerskjöld, 1903 : 14 Nicoll, 1907 : 483, 484 Poche, 1926 : 147 *Pres.	
DEXIOGONIMUS	DEXIOGONIMUS	Pres.	

Valid name	Described as	Author	Host
<i>Dexiogonimus ciurcanus</i>	<i>Dexiogonimus ciurcanus</i>	Pres.	<i>Felis catus dom.</i> , <i>Canis familiaris</i> , <i>Larus</i> sp.; metacercariae in <i>Tilapia simonis</i> , <i>T. galilea</i> , <i>Discognathus</i> sp., <i>Barbus canis</i> , <i>Mugil cephalus</i> , <i>M. capito</i> and <i>Licbia glauca</i> .
DIORCHITREMA	DIORCHITREMA	Pres.	
<i>Diorchitrema pseudocirrata</i>	<i>Diorchitrema pseudocirrata</i>	Pres.	<i>Felis catus dom.</i> , <i>Canis familiaris</i> ; metacercariae in <i>Mugil cephalus</i> , <i>M. capito</i> .
GALACTOSOMUM	GALACTOSOMUM	Looss, 1899: 671 Looss, 1902: 512 Pratt, 1902: 89c, 91c Jägerskjöld, 1908: 316, 317 Odhner, 1910: 354, 356 Pratt, 1911: 143-148 Nicoll, 1923a: 168 Poche, 1926: 152 Pres.	
<i>Galactosomum erinaceum</i>	<i>Galactosomum erinaceum</i>	Jägerskjöld, 1908: 317 Nicoll, 1923a: 240, 244 Pres.	
	<i>Distomum erinaceum</i>	Poirier, 1886: 37, 38, fig. 6 Braun, 1893: 643-870 Monticelli, 1893: 83, 86, 105, 106, 107 Looss, 1899: 590 Jägerskjöld, 1908: 317	<i>Delphinus delphis</i>
<i>Galactosomum lacteum</i>	<i>Galactosomum lacteum</i>	Looss, 1899: 671 Looss, 1902b: 512 Jägerskjöld, 1908: 316-317 Odhner, 1910: 354-356 Odhner, 1911: 181 Nicoll, 1915: 349, 354, 364 Nicoll, 1923a: 168, 179 Pres.	<i>Phalacrocorax carbo</i> Metacercariae in <i>Cottus bubalis</i>
	<i>Distomum hemiciclum</i> (?)	Molin, 1859: 829, 830 Carus, 1885: 127 Stossich, 1886: 43 Odhner, 1911: 186 Poche, 1926: 152 Pres.	<i>Belone acus</i> .
	<i>Monostomum lacteum</i>	Jägerskjöld, 1896: 165, 167-177, figs. 1-8, text-fig. 1 Jägerskjöld, 1899: 15 Looss, 1899: 671 Braun, 1899a: 724 Jägerskjöld, 1900: 736 Braun, 1901a: 47 Ward, 1901: 180 McLaren, 1904: 583 Jägerskjöld, 1908: 316	Metacercariae in <i>Cottus scorpius</i>

Valid name	Described as	Author	Host
HAPLORCHINAE	HAPLORCHINAE	Poche, 1926 : 152 Pres.	
	HAPLORCHINAE	Looss, 1899 : 671 *Poche, 1926 : 152	
	HAPLORCHIDINAE	Pratt, 1902 : 890 *Poche, 1926 : 152	
	MONORCHITREMINAE	Nishigori, 1924 Faust & N., 1926 : 124 *Pres.	
HAPLORCHIS	HAPLORCHIS	Looss, 1899 : 670, 671 Looss, 1902b : 442, 512 Pratt, 1902 : 890, 910 McCallum, 1902 : 636 Poche, 1926 : 152 Pres.	
<i>Haplorchis cabirinus</i>	<i>Haplorchis cabirinus</i>	Looss, 1899 : 671, 752-754, fig. 89 Odhner, 1910 : 355 Pres.	<i>Bagrus docmar</i>
	<i>Distomum cabirinum</i>	Looss, 1896b : 110-112, figs. 83, 84 *Looss, 1899 : 752	<i>Bagrus bayad</i>
<i>Haplorchis pumilio</i>	<i>Haplorchis pumilio</i>	Looss, 1899 : 671, 753 Pres.	
	<i>Monostomum pumilio</i>	Looss, 1896b : 154-158, figs. 101-106 *Looss, 1899 : 670, 753 Ciurea, 1924 : 3 Poche, 1926 : 152	<i>Pelecanus onocrotalus</i> , <i>Mikrus aegyptius</i>
HETEROPHYEA	HETEROPHYEA	Pres.	
HETEROPHYES	HETEROPHYES	Cobbold, 1866 : 6 Stiles & H., 1900 : 563 Looss, 1902a : 886 Looss, 1902b : 786, 805, 808, 824 Odhner, 1914 : 244 Skrjabin, 1919 : 13 Ransom, 1920 : 527, 529, 530 Nicoll, 1923b : 239 Ciurea, 1924 : 17 Stunkard & H., 1924 : 6 Faust & N., 1926 : 91 Pres.	
	COENOGONIMUS	Looss, 1899 : 585, 586, 619 Looss, 1900 : 608 Ofenheim, 1900 : 183 Jägerskjöld, 1900 : 736 Odhner, 1900 : 21, 21 Lühe, 1900b : 557 *Stiles & H., 1900 : 563	

Valid name	Described as	Author	Host
HETEROPHYES	COENOGONIMUS	Braun, 1901a : 56 Braun, 1901b : 334 Braun, 1901d : Looss, 1902a : 886 Looss, 1902b : 832, 835 Jägerskjöld, 1903 : 10, 11, 13, 15	
	COTYLOGONIMUS	Lühe, 1899 : 538, 539 Lühe, 1900b : 555, 557 Looss, 1900 : 607 Braun, 1900 : 6 Braun, 1900d : 56 *Stiles & H., 1900 : 563 Braun, 1901b : 334, 338 Looss, 1902b : 813, 833 Pratt, 1902 : 888 Stunkard & H., 1924 : 6	
	<i>Heterophyes aequalis</i>	Looss, 1902a : 888 Hall & Wigdor, 1918 : 237 Ransom, 1920 : 531, 535, 536 Skrjabin, 1923 : 4 Nicoll, 1923b : 239, 245, 246 Ciurea, 1924 : 13, 17 Pres.	<i>Felis catus dom.</i> , <i>Canis familiaris</i> . Persian wolf; metacercariae in <i>Mugil cephalus</i> , <i>M. capito</i> , <i>M. auratus</i> , <i>Epinephelus enaeus</i> , <i>Tilapia simonis</i> , <i>Lichia amia</i> , <i>L. glauca</i> and <i>Barbus canis</i>
	<i>Distomum fraternum</i> , partim	Looss, 1894a : 42-48, figs. 13-15 Looss, 1896b : 60-63, 101, 154, 156, figs. 36-37 Sonsino, 1896b : 314 Mühling, 1898b : 81-82 Stossich, 1898b : 42 Looss, 1899 : 535, 550, 556 Jägerskjöld, 1899 : 9, 12 Lühe, 1899 : 539 Jacoby, 1899 : 23 Braun, 1901b : 334, 336, 338 Looss, 1902a : 886-887 *Pres.	
	<i>Heterophyes inops</i>	Looss, 1902a : 887-888 Ransom, 1920 : 535 Ciurea, 1924 : 13, 17 *Pres.	<i>Pelecanus onocrotalus</i> , <i>Milvus aegyptius</i> .
<i>Heterophyes dispar</i>	<i>Heterophyes dispar</i>	Looss, 1902a : 888, 890, 891 Hall & Wigdor, 1918 : 237 Ransom, 1920 : 536-537 Skrjabin, 1923 : 4 Nicoll, 1923b : 239, 245, 246 Ciurea, 1924 : 13, 17 Pres.	<i>Felis catus dom.</i> , <i>Canis familiaris</i> Persian wolf; metacercariae in <i>Mugil cephalus</i> , <i>M. capito</i> , <i>M. auratus</i> , <i>Epinephelus enaeus</i> , <i>Tilapia simonis</i> , <i>Lichia amia</i> , <i>L. glauca</i> and <i>Barbus canis</i>

Valid name	Described as	Author	Host
<i>Heterophyes heterophyes</i>	<i>Cotylogonimus fraternus</i> , partim	Lühe, 1899 : 539 Braun, 1901b : 337 Fischöeder, 1903 : 548 Ransom, 1920 : 534 *Pres.	
	<i>Cotylogonimus heterophyes</i>	Lühe, 1899 : 539 Braun, 1901b : 335, 337, 338 Fischöeder, 1903 : 548 *Looss, 1902a : 886	
	<i>Cotylogonimus persicus</i>	Braun, 1901b : 334-338, fig. 13 Braun in Looss, 1902a : 891 Ransom, 1920 : 537 *Pres.	'Persian wolf.'
	<i>Dicrocoelium heterophyes</i>	Weinland, 1858 : 86 Cobbold, 1856 : 6 *Stiles & H., 1908 : 151	
	<i>Distoma fraternum</i> , partim	Looss, 1894a : 42-48, figs. 13-15 Mühling, 1898b : 81, 82 Looss, 1896b : 60-63, 101, 154, 156, figs. 36-37 Looss, 1899 : 535, 550, 556 Jacoby, 1899 : 23 Jägerskjöld, 1899 : 9, 12 Lühe, 1899 : 539 Braun, 1901b : 334, 336, 338 Looss, 1902a : 886, 887 *Pres.	<i>Pelecanus onocrotalus</i>
	<i>Distoma heterophyes</i> and <i>Distoma heterophyes</i> <i>hominis</i>	For the exhaustive references, see : Stiles & H., 1908 : 199 ; note *Stiles & H., 1900 : 563	
	<i>Fasciola heterophyes</i>	Moquin-Tandon, 1860 : 343 *Ransom, 1920 : 531	
	<i>Heterophyes aegyptiaca</i>	Cobbold, 1866 : 6 Stiles & H., 1900 : 563	
	<i>Heterophyes fraternus</i> , partim	Looss, 1902a : 887, 888 Looss, 1902b : 785, 808, 809, 838, 854 Looss, 1907 : 488 Ransom, 1920 : 534, 535, 536, fig. 3 Nicoll, 1923b : 239, 245, 246 Ciurea, 1924 : 13, 17 *Pres.	
	<i>Heterophyes heterophyes</i> <i>senilis</i>	Looss, 1902a : 890, 891 Ransom, 1920 : 533 *Pres.	
	<i>Heterophyes pallidus</i>	Looss, 1902a : 889, 890 Ransom, 1920 : 533, 534 Ciurea, 1924 : 17 *Pres.	<i>Milvus aegyptius</i>

Valid name	Described as	Author	Host
<i>Heterophyes heterophyes</i>	<i>Heterophyes persicus</i>	Braun in Looss, 1902a : 891 Looss, 1902b : 782, 785 Ransom, 1920 : 537, fig. 4 Poche, 1926 : 147 Cram, 1926 : 43 *Pres.	
	<i>Mesogonimus heterophyes</i>	Raillet, 1890 : 143 Stossich, 1892b : 31, 32 Monticelli, 1893 : 89, 155, 156 Ward, 1895 : 328 Blanchard, 1900 : 488	
<i>Heterophyes nocens</i>	<i>Heterophyes nocens</i>	Onji, 1915 : 875-883 Onji & Noshio, 1915 : Cort, 1921 : 187 Cort & Yokogawa, 1921 : 66-69, figs. 1-5 Lane, 1922 : 505 Ciurea, 1924 : 13, 17 Stunkard & H., 1924 : 2 Katsurada, 1925 : 2 Faust & N., 1926 : 92, 122 Nicoll, 1928 : 345 Pres.	Man; metacercariae in <i>Mugil cephalus</i> , <i>M. japonicus</i>
	<i>Heterophyes heterophyes</i> , part.	Janson & Tokishige, 1892 : 350 Janson, 1893 : 265 Leiper, 1913a : 176 Nicoll, 1928 :	<i>Canis familiaris</i>
	<i>Heterophyes katsuradai</i>	Ozaki & Azada, 1926 : 216-218, fig. 1 Azada, 1926 : 360-365, figs. 1-4 Nicoll, 1928 : 345, 376 *Pres.	
	<i>Mesogonimus heterophyes</i>	Raillet, 1890 : 143 Stossich, 1892b : 31-32 Ransom, 1920 : 531 *Ciurea, 1924 : 13 Faust & N., 1926 : 122	
HETEROPHYIDAE	HETEROPHYIDAE	Odhner, 1914 : 224 Skrjabin, 1919 : 13 Ransom, 1920 : 528-529 Travassos, 1921 : 85 Nicoll, 1923a : 168 Nicoll, 1923b : 238 Ciurea, 1924 : 16, 18 Stunkard & H., 1924 : 6 Dollfus, 1925 : 192 Faust & N., 1926 : 91, 92, 121 Poche, 1926 : 147, 163 Stiles & H., 1926 : 90, 91 Chapin, 1926 : 37 Pres.	
	APLORCHIDAE	Viana, 1924 : 107, 149 *Poche, 1926 : 147	

Valid name	Described as	Author	Host
HETEROPHYIDAE	COENOGONIMIDAE	Nicoll, 1907: 261 *Poche, 1926: 147	
	COTYLOGONIMIDAE	Nicoll, 1907: 261 *Poche, 1926: 147	
	HAPLORCHIDAE	Travassos in Viana, 1924: 159 *Poche, 1926: 147	
	STICTODORIDAE	Poche, 1926: 156 *Pres.	
HETEROPHYINAE	HETEROPHYINAE	Ciurea, 1924: 17 Stunkard, 1924: 6 Dollfus, 1925: 195 Pres.	
	COENOGONIMINAE	Looss, 1899: 586, 610 Odhner, 1900: 21, 23 Odhner, 1905: 314 Nicoll, 1909: 484 Stunkard & H., 1924: 6	
	COTYLOGONIMINAE	Pratt, 1902: 888 *Stunkard & H., 1924: 6	
	METAGONIMINAE	Ciurea, 1924: 17 Stunkard & H., 1926: 6 Stiles & H., 1926: 91	
METAGONIMUS	METAGONIMUS	Katsurada, 1912c Yokogawa, 1912a Yokogawa, 1912b Yokogawa, 1913b Ciurea, 1915b: 112 Skrjabin, 1919: 13 Ransom, 1920: 527, 529, 538, 539 Leiper, 1922: 364-365 Ciurea, 1924: 3, 4, 17 Stunkard & H., 1924: 6 Nicoll, 1924: 131 Dollfus, 1925: 195 Poche, 1926: 147 Stiles & H., 1926: 90, 91, 92 Nicoll, 1928: 345 Pres.	
	LOOSSIA	Ciurea, 1915a: 454-455 Ciurea, 1915b: 112 *Ransom, 1920: 527, 540 Ciurea, 1924: 4	
	LOXOTREMA	Kobayashi, 1912a: 785 Kobayashi, 1912b: 607 Leiper, 1922: 364, 365 Nicoll, 1923b: 239 *Dollfus, 1925: 195, 196 Faust & N., 1926: 92, 123 Stiles & H., 1926: 92 Poche, 1926: 147	

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METAGONIMUS	YOKOGAWA	Leiper, 1913 : 282 *Ransom, 1920 : 527, 539 Faust & N., 1926 : 123 Stiles & H., 1926 : 90, 92	
<i>Metagonimus romanicus</i>	<i>Metagonimus romanicus</i>	Ciurea, 1924 : 4, 5-10, 17 Stunkard & H., 1924 : 2 Pres.	
	<i>Loossia dobrogiensis</i>	Ciurea, 1915a : 454 Ciurea, 1915b : 108-112 *Ransom, 1920 : 38, 42-43 Ciurea, 1924 : 3-4	<i>Pelecanus onocrotalus</i>
	<i>Loossia parva</i>	Ciurea, 1915a : 453-454, fig. 6 Ransom, 1920 : 538, 541-542, fig. 10 *Ciurea, 1924 : 4	<i>Felis catus dom.</i> ; metacercariae in <i>Esax lucius</i>
	<i>Loossia romanica</i>	Ciurea, 1915a : 446-453, figs. 1-3, text-figs. 1-3 Ciurea, 1915b : 109-112 *Ransom, 1920 : 538, 541, figs. 8, 9 Hall & Wigdor, 1918 : 237 Ciurea, 1924 : 4 Stiles & H., 1926 : 90, 92	<i>Felis catus dom.</i> , <i>Canis familiaris</i> , <i>Sus scrofa dom.</i> , <i>Pelecanus onocrotalus</i> ; metacercariae in <i>Esax lucius</i> , <i>Scardinius erythrophthalmus</i> , <i>Abramis brama</i> , <i>Carassius carassius</i> , <i>Aspius aspius</i>
	<i>Metagonimus dobrogiensis</i>	Ciurea, 1924 : 3-4, 13, 17 *Pres.	
	<i>Metagonimus parvus</i>	Skrjabin, 1923 : 4 *Ciurea, 1924 : 4	
	<i>Metagonimus yokogawai</i> (partim)	Nicoll, 1924 : 129, 135, 136, 137, 138 Faust & N., 1926 : 123	
<i>Metagonimus yokogawai</i>	<i>Metagonimus yokogawai</i>	Katsurada, 1912c Yokogawa, 1912a Yokogawa, 1912b Katsurada, 1913 : 49-77, figs. 1-15 Yokogawa, 1913b Yokogawa, 1913d : 158-179, figs. 1-17 Ciurea, 1915b : 108-112 Muto, 1917b : 115 Muto, 1917d : 79 Ransom, 1920 : 538-543, figs. 5-9 Cort, 1921 : 187 *Leiper, 1922 : 364-365 Ando, 1922 : 1, 2, 4-9, 21, 22 Ciurea, 1924 : 4, 7, 8-10, 17 Nicoll, 1924 : 131, 135, 136, 137, 138 Stunkard & H., 1924 : 2 Dollfus, 1925 : 195-196 Faust & N., 1926 : 92, 115-116, 120 [Stiles & H., 1926 : 92-93 Nicoll, 1928 : 339, 343, 344, 345, 346 Pres.	Metacercariae in <i>Carassius aureus</i> , <i>Leuciscus baluensis</i> Cercariae in <i>Melania libertina</i> <i>Chimarrigale platycephala</i> , <i>Mus molossinus</i> , <i>Rattus rattus</i> , <i>Rattus norvegicus</i> Record following molluscan hosts: <i>Katayama nosophora</i> , <i>Melania ebenina</i> , <i>M. extensa</i> , <i>M. gottschei</i> , <i>M. nodiparadquinaria</i> , <i>M. obliquigranosa</i> , <i>Pyradus cingulatus</i>

Valid name	Described as	Author	Host
<i>Metagonimus yokogawai</i>	<i>Metagonimus yokogawai</i> (partim)	Nicoll, 1924 : 129 Faust & N., 1926 : 123	Man, <i>Felis catus dom.</i> , <i>Canis familiaris</i> ; metacercariae in <i>Plecoglossus altivelis</i>
	<i>Heterophyes elliptica</i>	Yokogawa, 1913a	
	<i>Heterophyes yokogawai</i>	Katsurada, 1912a Katsurada, 1912b *Dollfus, 1925 : 195-196	
	<i>Loxotrema ovatum</i>	Kobayashi, 1912a : 785 Kobayashi, 1912b : 607 Leiper, 1922 : 364-365 Nicoll, 1923b : 239, 246 *Dollfus, 1925 : 196	
	<i>Metagonimus ovatus</i>	Yokogawa, 1913c : 45-49, figs. 1, 2 *Ransom, 1920 : 538, 540, fig. 7 Poche, 1926 : 148	
	<i>Tacotrema yokogawa</i>	Leiper, 1913b : 282	
	<i>Yokogawa yokogawa</i>	Leiper, 1913a : 176 Leiper, 1913b : 282 *Ransom, 1920 : 538 Stiles & H., 1926 : 90, 92	
MICROLISTRUM	MICROLISTRUM	Braun, 1901c : 563, 895 Braun, 1902 : 55 Pratt, 1902 : 889 Odhner, 1910 : 354, 356 Pratt, 1911 : 143 Nicoll, 1923a : 168 Poche, 1926 : 152 Pres.	
	GALACTOSOMUM	Pratt, 1911 : 143-148 Poche, 1926 : 152 *Pres.	
<i>Microlistrum coelear</i>	<i>Microlistrum coelear</i>	Braun, 1901c : 563 Braun, 1902 : 56-58, fig. 36 Odhner, 1910 : 353-356, fig. 1 Nicoll, 1923a : 168, 192 Pres.	<i>Sterna sandwichensis</i> , <i>S. minuta</i>
	<i>Distomum coelear</i>	Diesing, 1850 : 357, 358 Creplin, 1851 : 288 Cobbold, 1860 : 14 Stossich, 1892a : 179 *Braun, 1901c : 561, 563, 895 Braun, 1902 : 56, 58	
	<i>Distomum coeleariforme</i> (partim)	Rudolphi, 1819 : 681 Dujardin, 1845 : 449 *Braun, 1901 : 893, 895 Braun, 1902 : 55, 56, 58	
	<i>Distomum coeleariforme sterna</i> (partim)	Diesing, 1850 : 357 Cobbold, 1860 : 14	

Valid name	Described as	Author	Host
<i>Microlistrum coeblear</i>	<i>Distoma diesingi</i>	Cobbold, 1860 : 14 *Stossich, 1892 : 179 Braun, 1901c : 561, 563 Braun, 1902 : 56, 58	
	<i>Galactosomum coeblear</i>	Pratt, 1911 : 143, 148 Viana, 1924 : 106, 107, 159	<i>Sterna antillarum</i>
<i>Microlistrum coebleari-forme</i>	<i>Microlistrum coebleari-forme</i>	Braun, 1901c : 563 Braun, 1902 : 56, 58, fig. 35 Odhner, 1910 : 353-356 Nicoll, 1923 : 168, 192 Pres.	
	<i>Distomum coebleariforme</i> (partim)	Rudolphi, 1819 : 681-682, 687 Dujardin, 1845 : 449 Diesing, 1850 : 357 Stossich, 1892a : 179 *Braun, 1901c : 561, 563, 895 Braun, 1902 : 55, 56	<i>Fregata aquila</i>
	<i>Distomum coebleariforme sternae</i> (partim)	Diesing, 1850 : 357 Cobbold, 1860 : 14	
	<i>Galactosomum coebleari-forme</i>	Pratt, 1911 : 143, 148 Viana, 1924 : 107, 159 *Pres.	
<i>Microlistrum semifuscum</i>	<i>Microlistrum semifuscum</i>	Pres.	
	<i>Galactosomum semifuscum</i>	Jägerskjöld, 1906 : 316, 317 Nicoll, 1923a : 168 *Pres.	
	<i>Monostomum semifuscum</i>	Olsson, 1876 : 28, figs. 65, 66 Brandes, 1892 : 505, 509 Monticelli, 1892 : 706, 710 Braun, 1893 : 916 *Jägerskjöld, 1908 : 317 Odhner, 1910 : 356	<i>Sula basana</i>
<i>Microlistrum spinctum</i>	<i>Microlistrum spinctum</i>	Braun, 1901c : 563, 895 Braun, 1902 : 59-62, figs. 37-39 Odhner, 1910 : 353, 355, 356	<i>Rhynchops nigra</i>
	<i>Galactosomum coebleari-forme</i>	Linton, 1928 : 23, fig. 52 *Pres.	<i>Fregata magnificens</i>
	<i>Galactosomum spinctum</i>	Viana, 1924 : 149, 159 *Pres.	
MONORCHITREMA	MONORCHITREMA	Nishigori, 1924 : 569 Faust & N., 1925 Faust & N., 1926 : 93 Pres.	

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<i>Monorchitrema taibokui</i>	<i>Monorchitrema taibokui</i>	Nishigori, 1924 : 569 Faust & N., 1925 Faust & N., 1926 : 93-125, figs. 1-5, 12-19, 25, 27, text-fig. 1 Nicoll, 1928 : 340, 345, 346	Molluscan hosts : <i>Melania reiniana</i> var. <i>bidacbiensis</i> ; secondary hosts : <i>Carassius auratus</i> , <i>Clarias fuscus</i> , <i>Cbanna formosana</i> , <i>Pseudasbora parva</i> , <i>Pboedcus ocellatus</i> , <i>Gambusia affinis</i> , <i>Polycantbus operculatus</i> , <i>Ctenophalus tadinus</i> , <i>Misgurnus anguillicaudatus</i> , <i>Parasilurus asotus</i> , <i>Zacco platypus</i> , <i>Cyprinus carpio</i> ; definite hosts : <i>Nycticorax nycticorax</i> , <i>Felis catus dom.</i> , <i>Canis familiaris</i> , rabbit, mouse, guinea-pig.
		Pres.	Metacercariae in <i>Tilapia nilotica</i> , <i>T. galilea</i> , <i>T. simonis</i> , <i>Barbus longiceps</i> , <i>Mugil capito</i> , <i>M. cepbalus</i> , <i>Barbus canus</i> ; adults in <i>Larus</i> sp.
<i>Monorchitrema taibui</i>	<i>Monorchitrema taibui</i>	Nishigori, 1924 : 569 Faust & N., 1925 Faust & N., 1926 : 93-125, figs. 2, 6-11, 20-22, 26, 28	Cercariae in <i>Melania obliquegranosa</i> ; metacercariae in <i>Cyprinus carpio</i> , <i>Carassius auratus</i> , <i>Zacco platypus</i> , <i>Pseudasbora parva</i> , <i>Pboedcus ocellatus</i> , <i>Gambusia affinis</i> , <i>Ctenopharyngodon idellus</i> ; adults in 'birds, mammals, including man.'
		Nicoll, 1928 Pres.	<i>Felis catus dom.</i> , <i>Canis familiaris</i> , <i>Larus</i> sp. ; metacercariae in <i>Varicorbinus</i> sp., <i>Barbus canus</i> , <i>Tilapia simonis</i> .
PARASCOCOTYLE	PARASCOCOTYLE	Stunkard & H., 1924 : 3, 4 Dollfus, 1925 : 192 Pres.	
	ASCOCOTYLE (partim)	Looss, 1899 : 584, 585, 586, 611 Braun, 1902 : 30 Looss, 1902b : 441, 824, 832, 833 Pratt, 1902 : 888, 894 Jägerskjöld, 1903 : 14 Nicoll, 1907 : 521 Odhnér, 1914 : 224 Skrjabin, 1919 : 13 Ransom, 1920 : 529, 561, 562 Nicoll, 1923a : 168 Nicoll, 1923b : 239 Ciurea, 1924 : 17 *Stunkard & H., 1924 : 2, 3, 6, 7 Pres.	
	PHAGICOLA	Faust, 1920 : 525, 531 *Faust & N., 1925 : 93 Poche, 1926 : 152, 153	

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<i>Parascocotyle ascolonga</i>	<i>Parascocotyle ascolonga</i>	Pres.	<i>Felis catus dom.</i> , <i>Canis familiaris</i> ; metacercariae in <i>Tilapia simonis</i> .
<i>Parascocotyle italica</i>	<i>Parascocotyle italica</i>	Stunkard & H., 1924 : 3 Pres.	
	<i>Ascocotyle italica</i>	Alessandrini, 1906 : 221-224 Hall & Wigdor, 1918 : 237 Ransom, 1920 : 262, 263, 264, 268 Nicoll, 1923 <i>b</i> : 239, 246 Skrjabin, 1923 : 4 Ciurea, 1924 : 14, 17 *Stunkard & H., 1924 : 3	<i>Canis familiaris</i>
	<i>Echinostomum pyriforme</i>	Nicoll, 1923 <i>b</i> : 239 Blanc & Hedin, 1913 *Skrjabin & Lindtrop, 1919 : 6	
<i>Parascocotyle longa</i>	<i>Parascocotyle longa</i>	Stunkard & H., 1924 : 3 Pres.	<i>Felis catus dom.</i> , <i>Canis familiaris</i> , Persian wolf; metacercariae in <i>Mugil cephalus</i> , <i>M. capito</i> , <i>Lichia amia</i> , <i>Barbus canis</i> .
	<i>Ascocotyle longa</i>	Ransom, 1920 : 564-566, fig. 29 Ciurea, 1924 : 14, 17 *Stunkard & H., 1924 : 3 Cram, 1926 : 43	<i>Vulpes lagopus</i>
<i>Parascocotyle minuta</i>	<i>Parascocotyle minuta</i>	Stunkard & H., 1924 : 3 Dollfus, 1925 : 192 Pres.	
	<i>Ascocotyle minuta</i>	Looss, 1899 : 585, 698-699, 700, 701, fig. 23 Looss, 1901 <i>a</i> : 205 Faria, 1910 : 287 Railliet & Henry, 1913 : 930 Travassos, 1916 : 1 Hall & Wigdor, 1918 : 237 Ransom, 1920 : 532, 538, fig. 28 Nicoll, 1923 <i>a</i> : 168, 185 Nicoll, 1923 <i>b</i> : 239, 246 Skrjabin, 1923 : 4 Joyeux, 1924 : 3 Viana, 1924 : 134, 157 Ciurea, 1924 : 14, 17 *Stunkard & H., 1924 : 3 Dollfus, 1925 : 192	<i>Felis catus dom.</i> , <i>Canis familiaris</i> , <i>Ardea cinerea</i>
	<i>Parascocotyle diminuta</i>	Stunkard & H., 1924 : 4-5, fig. 1 Dollfus, 1925 : 192-194, fig. 1 *Pres.	<i>Rattus norvegicus</i>
<i>Parascocotyle nana</i>	<i>Parascocotyle nana</i>	Stunkard & H., 1924 : 3 Pres.	
	<i>Ascocotyle nana</i>	Ransom, 1920 : 562, 566-568, fig. 30 Ciurea, 1924 : 14, 17 *Stunkard & H., 1924 : 3 Cram, 1924 : 3	<i>Vulpes lagopus</i>

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<i>Rossicotrema donicum</i>	<i>Cotylophallus similis</i>	Ransom, 1920 : 555, 558, 559, fig. 26 Nicoll, 1923b : 240, 243 Ciurea, 1924 : 14 *Pres.	<i>Pboca vitulina</i>
	<i>Cotylophallus venustus</i>	Ransom, 1920 : 555, 558, 559, figs. 22-25 Nicoll, 1923b : 240, 246 Hall, 1923 : 14 Ciurea, 1924 : 14 Cram, 1926 : 43 *Pres.	<i>Felis catus dom.</i> , <i>Canis familiaris</i> , <i>Falpes lagopus</i> .
	<i>Rossicotrema simile</i>	Ciurea, 1924 : 14, 17 *Pres.	
	<i>Rossicotrema venustum</i>	Ciurea, 1924 : 14 *Pres.	
SCAPHANOCEPHALUS	SCAPHANOCEPHALUS	Jägerskjöld, 1903 : 1-16 Lühe, 1909 : 88, 89 Odhner, 1914 : 244 Skrjabin, 1916 : 13 Ransom, 1920 : 527, 528 Nicoll, 1923a : 168 Ciurea, 1924 : 1, 14, 18 Stunkard & Il., 1924 : 6 Poche, 1926 : 147 Faust & N., 1926 : 91 Pres.	
<i>Scaphanocephalus australis</i>	<i>Scaphanocephalus australis</i>	Johnston, 1917 : 188-195, text-figs. 1-5, figs. 1, 14 Ciurea, 1924 : 18 Pres.	<i>Haliæctus leucogaster</i>
<i>capbanocephalus expansus</i>	<i>Scaphanocephalus expansus</i>	Jägerskjöld, 1903 : 1-10, figs. 1-5 Lühe, 1909 : 89 Johnston, 1917 : 188, 195 Nicoll, 1923a : 168, 179 Ciurea, 1924 : 2, 14 Pres.	
	<i>Distoma expansum</i>	Monticelli, 1892 : 714	
	<i>Monostomum expansum</i>	Creplin, 1842 : 327-336 Dujardin, 1845 : 345, 346 Diesing, 1850 : 321 Brandes, 1892 : 508 Monticelli, 1892 : 685, 686, 694, 696, 700, 703, 713, 714 Braun, 1893 : 915 Jägerskjöld, 1901 : 979, 983 *Jägerskjöld, 1903 : 1	<i>Pandion buliaëtus</i>
	<i>Tocotrema expansum</i>	Jägerskjöld, 1901 : 979-981, fig. 1 Looss, 1902 : 706 Odhner, 1902 : 45 *Jägerskjöld, 1903 : 1	

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STAMNOSOMA	STAMNOSOMA	Tanabe, 1922 : 19 Faust & N., 1926 : 121-122 Stiles & H., 1926 : 93 Pres.	
<i>Stamnosoma armatum</i>	<i>Stamnosoma armatum</i>	Tanabe, 1922 : 19 Faust & N., 1926 : 93, 116, 123 Stiles & H., 1926 : 93 Nicoll, 1928 : 345, 346 Pres.	Man, <i>Nycticorax nycticorax</i> , and laboratory animals; metacercariae in cyprinoid fishes.
<i>Stamnosoma formosanum</i>	<i>Stamnosoma formosanum</i>	Nishigori, 1924a Nishigori, 1924b Faust & N., 1926 : 93, 116, 121, 122, 124 Stiles & H., 1926 : 93 Nicoll, 1928 : 345, 346 Pres.	Adults in man, <i>Felis catus</i> <i>dom.</i> , <i>Canis familiaris</i> , guinea-pig, rat; metacer- cariae in <i>Carassius auratus</i> , <i>Channa formosana</i> , <i>Clarias</i> <i>fuscus</i> , <i>Ctenopharyngodon</i> <i>idellus</i> , <i>Cyprinus carpio</i> , <i>Gambusia affinis</i> , <i>Misgurnus</i> <i>anguillicaudatus</i> , <i>Opibcephalus</i> <i>tadjanus</i> , <i>Parasilurus asotus</i> , <i>Pseudorasbora parva</i> , <i>Rhodeus</i> <i>ocellatus</i> , <i>Zacco platypus</i> .
STICTODORA	STICTODORA	Looss, 1899 : 671-672 Pratt, 1902 : 890, 910 Poche, 1926 : 156 Pres.	
<i>Stictodora saevakinensis</i>	<i>Stictodora saevakinensis</i>	Looss, 1899 : 754, 755, fig. 90 Pres.	<i>Larus</i> sp. <i>Felis catus dom.</i> , <i>Canis</i> <i>familiaris</i> , <i>Puffinus kubl</i> ; metacercariae in <i>Mugil</i> <i>cephalus</i> , <i>M. capito</i>
TOCOTREMA	TOCOTREMA, s.str.	Nicoll, 1909 : 483 Pres.	
	TOCOTREMA (partim)	Looss, 1899 : 585, 586, 619 Looss, 1900 : 608 Odhner, 1900 : 21, 22 Jägerskjöld, 1900 : 736 Lühe, 1900b : 557 Braun, 1901a : 56 Jägerskjöld, 1901 : 981, 982 Looss, 1902b : 833, 835 Jägerskjöld, 1903 : 13, 14 Odhner, 1914 : 244 Skrjabin, 1919 : 13	
	CRYPTOCOTYLE (partim)	see CRYPTOCOTYLE (partim)	
	DERMOCYSTIS	Stafford, 1905 : 682 •Ransom, 1920 : 544	
	HALLUM	Wigdor, 1918 : 254 •Ransom, 1920 : 527, 547, 548 Maplestone, 1922 : 455 Stiles & H., 1926 : 90	

Valid name	Described as	Author	Host
<i>Tocotrema ecbinata</i>	<i>Tocotrema ecbinata</i>	Pres.	
	<i>Cryptocotyle ecbinata</i>	Lühe, 1909 : 88 Nicoll, 1923a : 168, 179 *Pres.	
	<i>Monostoma ecbinatum</i>	Linstow, 1878 : 223, 224, fig. 6 Brandes, 1892 : 509 Monticelli, 1892 : 685, 661, 694, 697, 698, 699, 702, 705, 713, 714 Braun, 1892 : 570, 586, 915 *Lühe, 1909 : 88	<i>Pandion haliaetus</i>
<i>Tocotrema jejunum</i>	<i>Tocotrema jejunum</i>	Nicoll, 1907 : 248, 257-259 Nicoll, 1909 : 483, figs. 20, 21 Pres.	<i>Totanus calidris</i>
	<i>Cryptocotyle jejuna</i>	Ransom, 1920 : 544, 548-550, figs. 16-17 Nicoll, 1923a : 168, 188 Ciurea, 1924 : 1, 213, 18, fig. 1 *Pres.	<i>Larus argentatus californicus</i> , <i>Sterna hirundo</i> .
<i>Tocotrema lingua</i>	<i>Tocotrema lingua</i>	Looss, 1899 : 586 Jägerskjöld, 1900 : 736 Jägerskjöld, 1901 : 979-982 Odhner, 1902 : 45 Kowalewski, 1902 : 27 (9) Jägerskjöld, 1903 : 2, 13 Nicoll, 1906 : 514, 519 Nicoll, 1914 : 152 Nicoll, 1915 : 354 Poche, 1926 : 147 Pres.	<i>Larus atricapilla</i> Larvae in <i>Cottus scorpius</i>
	<i>Cryptocotyle americana</i>	Ciurea, 1924 : 14-15 *Pres.	
	<i>Cryptocotyle lingua</i>	Fischöeder, 1903 : 548 Nicoll, 1907 Lühe, 1909 : 88 Ciurea, 1915a : 446, 450 Linton, 1915 : 128, 134 Ransom, 1920 : 544-548, figs. 12-15	<i>Rissa tridactyla</i> Metacercariae in various fishes
		Hall, 1923 : 14 Maplestone, 1922 : 153-156, fig. 1 Nicoll, 1923a : 168, 186, 190, 191, 192 Nicoll, 1923b : 240, 243, 246 Ciurea, 1924 : 14, 15, 18 Stunkard & H., 1924 : 2 Poche, 1926 : 148 Stunkard, 1927 : 125 *Pres.	<i>Colymbus auritus</i> , <i>Gavia imber</i> , <i>Nycticorax nycticorax</i> , <i>Alca torda</i> , <i>Sterna dougalli</i> , <i>S. hirundo</i> , <i>Pooca vitulina</i> .
			<i>Rattus albus</i> , <i>Felis catus dom.</i>

Valid name	Described as	Author	Host
<i>Tocotrema lingua</i>	<i>Dermocystis ctenolabri</i>	Stafford, 1905 : 682 Linton, 1915 : 128, 134	
	<i>Distoma lingua</i>	Creplin, 1825 : 333, 347, 348 Creplin, 1837 : 310 Dujardin, 1845 : 448 Creplin, 1846 : 139 Diesing, 1850 : 343 Cobbold, 1860 : 11 Olsson, 1876 : 15 Stossich, 1892a : 158 Braun, 1892 : 568, 599, 721 Olsson, 1893 : 11 Monticelli, 1893 : 94 Kowalewski, 1896 : 252 Stossich, 1896 : 129 Stossich, 1898 : 41, 42 Lühe, 1899 : 539 Jägerskjöld, 1898 : 4, 14, 16, figs. 1-4 *Looss, 1899 : 586 Jacoby, 1900 : 23 Jägerskjöld, 1901 : 982 Jägerskjöld, 1903 : 1, 5	<i>Larus marinus</i> <i>Larus argentatus</i> <i>Larus fuscus</i>
	<i>Distoma</i> sp.	Ryder, 1844 : 37-42 Linton, 1898 : 281, 296, figs. 76, 81 Linton, 1891 : 462, 463, fig. 318 *Stafford, 1905 : 682 Linton, 1912 : 255, 257	Metacercariae in <i>Tautoglabrus adaspermus</i> .
	<i>Hallum caninum</i>	Wigdor, 1918 : 254-257, figs. 1-4 *Ransom, 1920 : 527, 547, 548 Maplestone, 1922 : 155 Hall, 1923 : 14	<i>Canis familiaris</i>

REFERENCE LIST OF LITERATURE CONCERNING CERCARIAE OF UNDETERMINED SPECIES OF *HETEROPHYIDAE*

Valid name	Described as	Author	Host
<i>Cercaria chromophila</i>	<i>C. chromophila</i>	Faust, 1922 : 262-263, fig. 18 Faust, 1924 : 292 Faust & N., 1926 : 124	<i>Melania obenina</i>
	<i>C. flavopunctata A</i>	Kobayashi, 1922 : 12-13, figs. 4, 5	<i>Blanfordia nosopora</i> , <i>Pyradus cingulatus</i> and different species of <i>Melania</i> .
<i>C. cordata</i>	<i>C. cordata</i>	Faust, 1924 : 254, 293, fig. 15 Faust & N., 1926 : 117, 120, 124	<i>Melanoides tuberculatus</i>
<i>C. pbatifera</i>	<i>C. pbatifera</i>	Faust, 1922 : 262, fig. 16 Faust, 1924 : 292	<i>Viviparus polysomatus</i>
<i>C. translucens</i>	<i>C. translucens</i>	Faust & N., 1926 : 117, 119, 124 Faust & N., 1926 : 118, 119, 124, fig. 23	<i>Bythinia striatula</i>
<i>C. tridonta</i>	<i>C. tridonta</i>	Faust & N., 1926 : 119-120, 124, fig. 24	<i>Bythinia sinensis</i>
<i>C. picta</i>	<i>C. picta</i>	Faust, 1924 : 292	
	<i>C. flavopunctata B</i>	Kobayashi, 1922 : 13, figs. 6, 7	<i>Melania libertina</i> , <i>M. reiniana</i> , <i>Melania</i> sp.

REFERENCE LIST OF SYNONYMA OF HETEROPHYIDEA.

Synonym	Valid name*
<i>Anoikistoma coleostoma</i>	<i>Ascocotyle coleostoma</i>
" <i>cuspidatum</i>	<i>Centrocestus cuspidatus</i>
APLORCHIDAE	HETEROPHYIDAE
APLORCHINAE	HAPLORCHINAE
<i>Apoballus brevis</i>	<i>Rossicotrema donicum</i>
" <i>major</i>	<i>Apoballus müblingi</i>
ASCOCOTYLE (partim)	PARASCOCOTYLE
" <i>italica</i>	" <i>italica</i>
" <i>longa</i>	" <i>longa</i>
" <i>minuta</i>	" <i>minuta</i>
" <i>nana</i>	" <i>nana</i>
" <i>pithecophagicola</i>	" <i>pithecophagicola</i>
" <i>plana</i>	<i>Pygidiopsis genata</i>
CIURFANA	CRYPTOCOTYLE
" <i>cryptocotyloides</i>	" <i>cryptocotyloides</i>
" <i>quinqueangularis</i>	" <i>quinqueangularis</i>
<i>Clinostomum heterophyes</i>	<i>Heterophyes heterophyes</i>
COENOGONIMUS	HETEROPHYES
" <i>fraternus</i>	" <i>heterophyes</i>
" <i>heterophyes</i>	" "
COENOGONIMIDAE	HETEROPHYIDAE
COENOGONIMINAE	HETEROPHYINAE
<i>Centrocestus cuspidatus</i> var. <i>caninus</i>	<i>Centrocestus cuspidatus</i>
COTYLOGONIMIDAE	HETEROPHYIDAE
COTYLOGONIMINAE	HETEROPHYINAE
COTYLOGONIMUS	HETEROPHYES
" <i>fraternus</i>	" <i>heterophyes</i>
" <i>heterophyes</i>	" "
" <i>persicus</i>	" "
COTYLOPHALLUS	ROSSICOTREMA
" <i>similis</i>	" <i>donicum</i>
" <i>venustus</i>	" "
CRYPTOCOTYLE (partim)	TOCOTREMA
" <i>americana</i>	" <i>lingua</i>
" <i>ecbinata</i>	" <i>ecbinata</i>
" <i>jejunum</i>	" <i>jejunum</i>
<i>Distomum cabirinum</i>	<i>Haplochebis cabirinus</i>
" <i>cochlear</i>	<i>Microlistrum cochlear</i>
" <i>cochleariforme</i> (partim)	" <i>cochleariforme</i>
" <i>cochleariforme sternae</i> (partim)	" <i>spinetum</i>
" <i>coleostomum</i>	" <i>cochlear</i>
" <i>colostomum</i>	" <i>cochleariforme</i>
" <i>concauum</i>	<i>Ascocotyle coleostoma</i>
" <i>cuspidatum</i>	" "
" <i>disringi</i>	<i>Cryptocotyle concauum</i>
" <i>erinaceum</i>	<i>Centrocestus cuspidatus</i>
" <i>expansum</i>	<i>Microlistrum cochlear</i>
" <i>fraternum</i> (partim)	<i>Galactosomum erinaceum</i>
" <i>bemiciclum</i>	<i>Scaphanocephalus expansum</i>
" <i>heterophyes</i>	<i>Heterophyes aequalis</i>
" <i>bominis</i>	" <i>dispar</i>
<i>Distomum lingua</i> (partim)	" <i>heterophyes</i>
" <i>müblingi</i>	<i>Galactosomum lacteum</i>
" sp. of Ryder, 1844	<i>Heterophyes heterophyes</i>
DERMOCYSTIS	" "
" <i>ctenolabri</i>	<i>Apoballus müblingi</i>
	<i>Tocotrema lingua</i>
	<i>Apoballus müblingi</i>
	<i>Tocotrema lingua</i>
	TOCOTREMA
	" <i>lingua</i>

*For particulars see the Reference List of Bibliography.

REFERENCE LIST OF SYNONYMA OF *HETEROPHYIDAE*—continued

Synonym	Valid name*
<i>Dicrocoelium heterophyes</i>	<i>Heterophyes heterophyes</i>
<i>Echinostomum pyriforme</i>	<i>Parascocotyle italica</i>
<i>Fasciola heterophyes</i>	<i>Heterophyes heterophyes</i>
<i>Galactosomum</i> (partim)	<i>Microlistrum</i>
" <i>cocblear</i>	" <i>cocblear</i>
" <i>cocbleariforme</i>	" <i>cocbleariforme</i>
" <i>semifuscum</i>	" <i>semifuscum</i>
" <i>spinetum</i>	" <i>spinetum</i>
HALLUM	TOCOTREMA
" <i>caninum</i>	" <i>lingua</i>
HAPLORCHIDAE	HETEROPHYIDAE
HAPLORCHIDINAE	HAPLORCHINAE
HAPLORCHINAE	"
<i>Heterophyes aegyptiacus</i>	<i>Heterophyes heterophyes</i>
" <i>dispar limatus</i>	" <i>dispar</i>
" <i>elliptica</i>	<i>Metagonimus yokogawai</i>
" <i>fraternus</i> (partim)	<i>Heterophyes aequalis</i>
" <i>heterophyes</i>	" <i>dispar</i>
" " <i>sentus</i>	" <i>heterophyes</i>
" " <i>inops</i>	" <i>nocens</i>
" <i>katsuradai</i>	Valid name (?)
" <i>pallidus</i>	<i>Heterophyes heterophyes</i>
" <i>persicus</i>	" <i>aequalis</i>
" <i>yokogawai</i>	" <i>nocens</i>
" " <i>heterophyes</i>	" <i>heterophyes</i>
LOOSSIA	" " <i>sentus</i>
" <i>dobrogensis</i>	Valid name (?)
" <i>parva</i>	<i>Heterophyes heterophyes</i>
" <i>romanica</i>	" <i>aequalis</i>
LOXOTREMA	" <i>nocens</i>
" <i>ovatum</i>	" <i>heterophyes</i>
<i>Mesogonimus heterophyes</i> (partim)	" " <i>sentus</i>
<i>Metagonimus dobrogensis</i>	Valid name
" <i>ovatus</i>	<i>Metagonimus romanicus</i>
" <i>parvus</i>	" <i>yokogawai</i>
" <i>yokogawai</i> (partim)	" <i>romanicus</i>
<i>Metorchis oscophagolongus</i>	<i>Metagonimus romanicus</i>
MONORCHITREMINAE	Valid name
<i>Alonostomum expansum</i>	<i>Apophallus müblingi</i>
" <i>lacteum</i>	HAPLORCHINAE
" <i>pumilio</i>	<i>Scaphanocephalus expansus</i>
" <i>semifuscum</i>	<i>Galactosomum lacteum</i>
<i>Parascocotyle diminuta</i>	<i>Haplorchis pumilio</i>
PHAGICOLA	<i>Microlistrum semifuscum</i>
" <i>pithecopbagicola</i>	<i>Parascocotyle minuta</i>
PHAGICOLINAE	PARASCOCOTYLE
<i>Rossicotrema simile</i>	" <i>pithecopbagicola</i>
" <i>venustum</i>	CENTROCESTINAE
STICTODORIDAE	<i>Rossicotrema donicum</i>
TOCOTREMA (partim)	" " <i>sentus</i>
" <i>concauum</i>	HETEROPHYIDAE
" <i>expansum</i>	CRYPTOCOTYLE (partim) Valid name
<i>Tocotrema müblingi</i>	" <i>cryptocotyle concauum</i>
" <i>yokogawa</i>	<i>Scaphanocephalus expansus</i>
YOKOGAWA	<i>Apophallus müblingi</i>
" <i>yokogawa</i>	<i>Metagonimus yokogawai</i>
" " <i>heterophyes</i>	METAGONIMUS
" " <i>sentus</i>	" <i>yokogawai</i>

*For particulars see the Reference List of Bibliography.

SUMMARY

All members of *Heterophyidae* are reclassified on a system of taxonomical coefficients. The family is divided into five sub-families: *Heterophyinae* Ciurea, *Centrocestinae* Looss, *Haplorchinae* Pratt, *Cercarioidinae* nov. subf., and *Adleriinae* nov. subf. The total number of genera reported is 21, of species 43. Four new genera are established: *Diorchitrema*, *Dexiogonimus*, *Cercarioides*, and *Adleria*. Fourteen species of the family *Heterophyidae* are reported from Palestinian hosts; among these the following five are new ones: *Parascocotyle ascolonga*, *Cercarioides aharonii*, *Adleria minutissima*, *Diorchitrema pseudocirrata*, and *Dexiogonimus ciureanus*. A number of specific and generic names are rejected as synonyms; a number of species hitherto regarded as belonging to the *Heterophyidae* is attributed to other systematic groups for reasons given in the text.

The development of members of the *Heterophyidae* in the final host was elucidated experimentally. It is found that, as a rule, fish are intermediary hosts of *Heterophyidae*, i.e., they harbour encysted metacercariae. The latter develop in the final host within seven to eight days. The species of fish which serve as secondary hosts of almost all Palestinian *Heterophyidae* are listed. *Inter alia*, 100 per cent. of mullets sold on the Palestinian markets are infected with the metacercariae of *Heterophyes* species and should be regarded as the source of human infection.

An almost full bibliography dealing with all specific, generic, etc., names of *Heterophyidae* and their synonyms is given.

For the purpose of comparison by other workers the following species are deposited in the United States National Museum, the Liverpool School of Tropical Medicine, the Zoological Museum of the Berlin University, Molteno Institute, Cambridge, and Helminthological Institute, Moscow.

Heterophyes heterophyes, from the dog.

Heterophyes heterophyes, from the cat.

Heterophyes dispar.

Heterophyes aequalis.

Dexiogonimus ciureanus.

Monorchitrema taihokui.

Monorchitrema taihui.

Stictodora sawakinensis.

Diorchitrema pseudocirrata.

Parascocotyle longa.

Adleria minutissima.

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A STUDY OF EXPERIMENTAL INFECTION BY *TREPONEMA DUTTONI*; . WITH A REVIEW OF THE LITERATURE

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INTRODUCTION

This paper records the results of a study of experimental infection by *Treponema duttoni*. Although the relation of the organism to Tick Fever in the human subject has for long been firmly established, the literature regarding the experimental disease contains numerous discrepancies among the observations made by different workers. While some of the results obtained by the writer are in agreement with observations already published, certain data elicited are contrary to those of other workers and, therefore, appear of sufficient importance to warrant their being recorded. The investigation has also been of interest in eliciting certain special characteristics of the strain of organism used—its varying pathogenicity for different animals, the possible avenues of infection, the absence of demonstrable antibodies in the serum of infected animals, the high resistance of the organism to organic arsenicals and the persistence of forms in the body after the apparent recovery of the host.

The strain of *Treponema duttoni* used was obtained from Dr. J. G. Thomson, of the London School of Hygiene and Tropical Medicine, and had been maintained for a considerable period in laboratory animals.

SUSCEPTIBLE AND REFRACTORY ANIMALS.

Experimental infection was produced in mice, rats, a young guinea-pig and two monkeys (*Macacus sinicus*). Without exception all the mice and rats inoculated developed the disease. In all 348 mice and 22 rats were infected. While Breinl and Kinghorn (1906) stated that young guinea-pigs were more susceptible than adult ones, it was

found that adult animals were altogether refractory. Other observers have found them refractory to various strains (Selwyn-Clarke, Le Fanu and Ingram, 1923, and Novy and Knapp, 1906). The latter were using a spirochaete which they termed *Spirochaeta obermeieri*, but which, according to Balfour (1911) and Cunningham (1925), was more probably a strain of *Treponema duttoni*. They believed that the scanty forms which they noted in the blood of one of three guinea-pigs inoculated two days previously, were probably mere survivals of those injected, but they examined the guinea-pigs only for ten days—an insufficient length of time, since the incubation period in the young guinea-pig successfully infected was sixteen days. Guinea-pigs, however, have been found to be susceptible to a Panama strain (Bates, Dunn and St. John, 1921), to a Russian strain (Nicolle and Anderson, 1927) and to a Spanish strain (Nicolle and Anderson, 1928).

Rabbits were refractory even when a large inoculum was injected intravenously into young animals. Novy and Knapp (1906), Toyota (1919) and Selwyn-Clarke, Le Fanu and Ingram (1923) likewise found them refractory, but they are stated to be susceptible to large doses (Breinl and Kinghorn), to intravenous injection with an arsenic-resistant African strain (Prigge, 1926), to suboccipital injection (Plaut, 1926), to inoculation into the cerebro-spinal fluid (Stremmel, 1928) and to Dakar strain (Mathis, 1927). The finding by Norris, Pappenheimer and Flournoy (1906) of forms in the blood of rabbits on the second day subsequent to inoculation but not thereafter was possibly due to survival of those in the inoculum as in the experiments of Novy and Knapp (*v. supra*).

INOCULUM.

The *inoculum* for the various experiments varied. While the blood of an infected animal was most often used, emulsions of various organs, such as of the spleen and brain and an emulsion of the embryos from an infected mother were also successfully employed.

AVENUES OF INFECTION.

Various modes of inoculation were investigated. All *subcutaneous* and *intraperitoneal* injections into susceptible mice and rats with material containing visible spirochaetes produced the infection. In addition it was found that infective material when rubbed into the

unbroken or scarified skin of mice could produce the infection. In one series of experiments great care was taken not to scarify the skin, and the mice kept separate so as to avoid injury by fighting. The hair over the back of each mouse was clipped and a barium sulphide depilating powder applied. Two days later blood containing large numbers of spirochaetes was rubbed into the depilated area with the gloved finger, and on four out of eight occasions infection resulted. Two mice became infected even when methylated spirit had been applied two minutes subsequent to the application of the infected blood. In no case, however, in which ether alone or iodine alone, or ether and then iodine had been applied subsequent to the inoculation, did infection result. This possibly depended on the penetration of the skin by the antiseptic. As recorded in a previous paper, several workers, in contradistinction to the results obtained by Werner (1924), state that infection can occur through the unbroken skin, and the writer, who himself became accidentally infected in the course of this work, believes that the organism gained access by this avenue (Gray, 1928). Moreover, the power of the spirochaetes to penetrate various tissue cells which do not exhibit any phagocytic action has been recorded by Strempel and Armuzzi (1927b).

Experimental infection was also produced by the avenue of certain *mucous membranes*. When a drop of infected blood was rubbed on to the nose of a healthy mouse, infection resulted in every case. Great care, of course, was taken not to injure the mucosa, the blood being placed in the concavity of a sterile hollow-ground slide and the animal's nose being gently rubbed into the concavity. Four mice *fed* with infected blood all developed the infection—the same result as that obtained by Feldt and Schott (1925) in 95 per cent. of cases. Infection by the *oral administration* of relapsing fever spirochaetes has also been produced in mice by Fraenkel (1907), Nattan-Larrier (1909) and Werner (1924), and in monkeys by F. P. Mackie (1907). Experiments similar to those of Gwélessiany (1927), were performed in which *Treponema duttoni* and *Trypanosoma brucei* were applied to the nasal mucosa, great care being taken to avoid incidental injury. Both organisms were found to be able to penetrate when applied either singly or in a mixture although, according to Gwélessiany, the spirochaetes alone enter.

The question of the *placental transmission* was also investigated.

Samples of blood of the young born of three infected mothers were examined for spirochaetes by the dark-ground method—all with negative results. Two of the litters were born just before the crisis terminating the first attack of each mother—when the blood of the latter contained very numerous spirochaetes. Inoculation of healthy mice with an emulsion of the body of a foetus belonging to one of these litters revealed the presence of the spirochaetes, but a foetus from the other litter was not infective. A mouse of the third litter, although born two and a half months subsequent to the last attack experienced by the mother, was proved by the inoculation of healthy mice to be infected. Since the inoculation of an apparently recovered mouse with trypanosomes sometimes causes the spirochaete to reappear in the circulating blood (see p. 258) attempts were made to demonstrate spirochaetes in the young born of an infected mother by inoculating them with *Trypanosoma brucei*. No spirochaetes, however, were demonstrated by this method.

The fact that a mouse born of an infected parent need not itself be infected indicates that the placenta may, as stated by Stempel and Armuzzi (1927a), have the power of hindering the passage of the spirochaetes and the infection of young mice recorded above need not, of course, have been conveyed via the placenta but during or subsequent to birth (see Miki, 1925). Nevertheless, Breinl and Kinghorn (1906), Nattan-Larrier (1911) and Leger and Bédier (1922) have all produced evidence in favour of the possibility of the passage of spirochaetes through the placenta.

Another fact elicited in this part of the work was that neither pregnant rats nor mice when infected showed any tendency to abortion. This observation is similar to that made by Breinl and Kinghorn (1906) in regard to rats.

INCUBATION PERIOD.

In computing the incubation period, daily examinations of the peripheral blood were made by the dark-ground method, and the day on which the organism was first observed was regarded as the first day of the attack. Determined in this way, the incubation period varied with the individual animal as well as with the species, the dosage, the avenue of infection and the administration of drugs.

TABLE I.

SHOWING THE VARIATIONS IN THE DURATION OF THE INCUBATION PERIOD, ATTACKS AND INTERVALS, AND IN THE NUMBER AND SEVERITY OF THE ATTACKS IN MICE.

The figures denote the result of daily observations on the tail-blood by the dark-ground illumination method, day 1 being the day on which inoculation was made.

o denotes failure to observe spirochaetes.

1 " the observation of at least 1 spirochaete in the drop of blood examined.

2 " the observation of at least 1 spirochaete in each field (magnification = 720).

3 " the observation of at least 5 spirochaetes in each field (magnification = 720).

4 " the observation of at least 10 spirochaetes in each field (magnification = 720).

5 " the observation of at least 40 spirochaetes in each field (magnification = 720).

D " death.

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Mouse 80	o	1	2	4	4	5	4	D
" 51	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	2	2	3	2	o	o	o	1	o	o	o	o	o	o
" 17	o	o	o	2	2	o	o	o	o	1	2	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
" 38	o	o	o	1	3	4	4	3	4	1	2	3	4	4	4	o	D
" 115	o	o	1	3	4	1	o	o	1	2	o	o	o	1	o	o	1	1	o	3	3	o	o	o	o	o	o	o	o	o
" 76	o	o	1	4	3	2	o	o	o	o	o	o	o	o	o	o	o	1	1	o	o	o	o	o	o	o	o	o	o	o
" 105	o	1	1	3	4	o	o	o	1	1	o	o	1	o	o	o	1	1	o	o	o	o	o	1	1	o	o	o	o	o
" 112	o	o	o	1	4	4	o	o	1	1	1	D

The incubation period varied between 1 day (mouse 80), and 16 days (mouse 51).

The duration of the first attack varied between 2 days (mouse 17), and 12 days (mouse 38).

The duration of the intervals varied between 2 days (mouse 115), and 11 days (mouse 76). (The arbitrary criterion of an interval was absence of the spirochaetes for two consecutive days.)

The number of attacks varied between 2 (mouse 17), and 5 (mouse 105).

The severity of the first attack varied between a maximum of 1 spirochaete in each field (mouse 17), and a maximum of 40 spirochaetes in each field, ending in death (mouse 80).

Death usually occurred during or immediately after the first attack (mice 38 and 80), but occasionally took place in the second attack (mouse 112).

In mice the incubation period varied between one and sixteen days (Table I). It tended to be shorter in young animals (cf. Feldt and Schott, 1925), when large doses were given and when subcutaneous and intraperitoneal inoculation was employed as compared with infection through the nasal mucosa, alimentary tract and especially the skin. Considerable variations were noted between individual mice. For instance, three healthy mice of the same weight were inoculated subcutaneously at the same time with equal doses of the same infected blood; the circulating blood of two was positive on the day following the inoculation while that of the third remained negative until the fourth day after inoculation.

TABLE II.

SUPERINFECTION.

Mice 60 to 65, of approximately equal weights, were inoculated at the same time with equal doses of the same infected material. On the fourth day mice 63 and 65 were again inoculated with infected material. They experienced longer attacks and the spirochaetes were more numerous in their blood than in mice 60-62. The latter also illustrate variations in the incubation period and in the duration and severity of the first attack, when equal doses are administered to animals of the same weight. (The figures are used as in Table I.)

Day	1	2	3	4	5	6	7	8	9	10
Mouse 60	0	0	0	0	3	3	3	0	1	0
" 61	0	1	2	4	4	2	1	0	1	0
" 62	0	1	3	4	4	0	0	1	1	1
" 63	0	1	2	4	4	5	2	1	2	4
" 64	0	1	2	4	5	4	2	0	1	2
" 65	0	0	1	3	4	5	3	1	0	0

It was also noticed that continued passage, especially when the sub-inoculations were made from mice in the first attack, tended slightly to shorten the incubation period and to enhance the virulence of the spirochaete for these animals—a finding similar to those of Collier (1925) and Plaut (1925). In contradistinction, however, to the work of these observers and also of Sagel (1928) the virulence for man was not lost or at least diminished, for the writer himself became accidentally infected in the course of these investigations (Gray, 1928).

Werner (1924) noted that in mice inoculated with *Treponema duttoni* in blood taken from the human subject during afebrile intervals, the incubation period was longest when the blood was taken on the first or second day of the interval.

In rats the incubation period varied between one and nine days, the longer periods being observed when large doses of 'sulfarsenol' were given intramuscularly or of '914' intravenously (*v. infra*).

In the young guinea-pig which was infected, the incubation was sixteen days.

The earliest observation of spirochaetes by the dark-ground illumination method in the peripheral blood of the first monkey was two days, and of the second monkey thirteen days after inoculation.

It is of interest, however, to note that of the mice which were inoculated at frequent intervals with the second monkey's blood, the first mouse to become infected was the one injected on the seventh day after the inoculation of the monkey.

FEATURES OF THE DISEASE

The features of the disease produced in the various animals varied considerably. Relapses were observed in mice, rats, and the monkeys. In differentiating one attack from another, absence of spirochaetes from the circulating blood for two consecutive days as shown by the dark-ground illumination method was arbitrarily taken as the criterion of an interval.

In *mice* the number of attacks noted varied between two and five (Table I). The condition proved fatal in 19 per cent. of the mice inoculated, but it was impossible to calculate the exact mortality due to the spirochaetal infection owing to the presence of inter-current disease such as mouse-typhoid. Most of the animals which succumbed did so in the first attack, but occasionally death occurred during the second or third attack. In contradistinction to the findings of Tomioka (1924) neither the percentage of deaths was higher nor the number of relapses greater after percutaneous than after subcutaneous or intraperitoneal inoculation. Of the mice which survived, one was found to harbour the organism in the circulating blood four and a half months subsequent to the date of inoculation. Persistence of infection of the blood in human cases has been recorded by Ehrlich, Weichbrodt (1920), Mayer (1922), and Werner (1924), and in a rat by Todd (1920).

PRESENCE OF THE SPIROCHAETES IN THE CIRCULATING BLOOD.

With the onset of the first attack in mice, the number of spirochaetes increased fairly rapidly and after remaining at a fairly constant high level for from four to twelve days decreased abruptly. At the height of the first attack, often as many as fifty organisms were present in each field of the dark-ground microscope (magnification = 720)—a much greater number than in human blood infected with *Treponema duttoni*. Not infrequently when the first attack of the mouse was of long duration—e.g., ten days or

more—there was for one or two days about the middle of the attack a very considerable decrease in the number of organisms present in the circulating blood (Table I).

An interval of from two to eleven days followed the first attack (Table I). Thereafter spirochaetes reappeared in the circulating blood. During such a remission although as judged by the dark-ground illumination method, the blood did not contain the organisms, it remained infective for healthy mice—a finding similar to those of Novy and Knapp (1906), Breinl and Kinghorn (1906), Weichbrodt (1921), Darling (1909), and Moselli (1923). The third and fourth attacks, each of which lasted for one to three days, occurred at intervals of one to eight days.

In contradistinction to Moczutkowsky (1882) it was found that the number of organisms (visible in the blood) in each successive attack became smaller. The statement of von Limbeck (1901) that the organisms are not uniformly distributed throughout the blood was confirmed for on several occasions they were found to be much more numerous in the heart blood than in blood taken from the tail.

In *rats*, the number of attacks noted never exceeded three. Buschke and Kroó (1923) found that white rats showed illness for weeks after inoculation and although the spirochaetes could not be demonstrated in their blood, inoculation of tissue emulsion into other animals, however, produced the disease. Toyota (1919) with a Manchurian strain obtained relapses in rats only after the 204th passage and Bates, Dunn, and St. John (1921) only occasionally obtained a relapse in rats. Selwyn-Clarke, Le Fanu and Ingram (1923), working with a Gold Coast strain, found that while black rats had only one attack, pouched rats had a relapse as well.

The young *guinea-pig* which was infected with a large amount of mouse-blood rich in spirochaetes showed for two days fairly numerous organisms in its circulating blood. It died on the third day of the disease.

The *monkeys* were inoculated with infected mouse blood subcutaneously. The spirochaetes were never numerous in the peripheral blood and, as judged by the dark-ground method, were not present for longer than a day at a time. Their numbers and certain of the blood changes are depicted in the charts. During each of its

two attacks the first animal was irritable and its skin became dry. The respirations were greatly increased, the alae nasi moving with each respiration and diarrhoea was fairly severe. The temperature and pulse were also increased. This monkey died on the thirty-seventh day of the experiment.

A good illustration of an abortive attack as described by Mayer (1922) and the writer (1928) is depicted in the chart between 11th and 13th December. Although no spirochaetes were observed on these dates the temperature, pulse, respirations, white blood count, and percentage of lymphocytes all showed distinct rises.

The second monkey did not present symptoms even on the four occasions on which spirochaetes were observed and it eventually survived the experiment only to succumb to pneumonia two months later. It is noteworthy that the temperature, pulse, and respirations were irregular, showing no constant deviations during the four attacks. By the inoculation of healthy mice it was shown that this monkey's blood continued to be infective for 44 days subsequent to inoculation :—thereafter it failed to produce the disease.

CHANGES IN THE BLOOD COUNT AND BLOOD PICTURE.

Daily observations were made on the blood of both monkeys (see charts). During the actual attacks there was a *diminution in the number of red blood corpuscles and in the haemoglobin*, but with each remission the number of red cells again rose though not quite to the previous level. Mayer (1922) states that this rise during convalescence is delayed if the spirochaetes persist in the blood. While reticulocytes were numerous there was no poikilocytosis or polychromatophilia as noted by Suldey (1920) in human cases in Madagascar and no evidence of the aplastic type of anaemia stated by Manson-Bahr to be especially noticeable in the African Tick Fever.

A *rise in the white blood count* was noted in connection with each attack, due to a marked increase in the number of lymphocytes (see charts). This change usually reached its maximum on the day preceding the attack but was occasionally delayed until the day of the attack. This is in keeping with the observation by Suldey of an increase of large mononuclears at the crisis but his statements as to a leucopenia and a relative lymphocytosis during the intervals were not confirmed. Karawacki and Krakowska (1921) state that the

CHART 1. — MALE MONKEY — INOCULATED SUBCUTANEOUSLY WITH TREPONEMA DUTTONI, 15th NOVEMBER, 1927. DIED 21st DECEMBER, 1927.

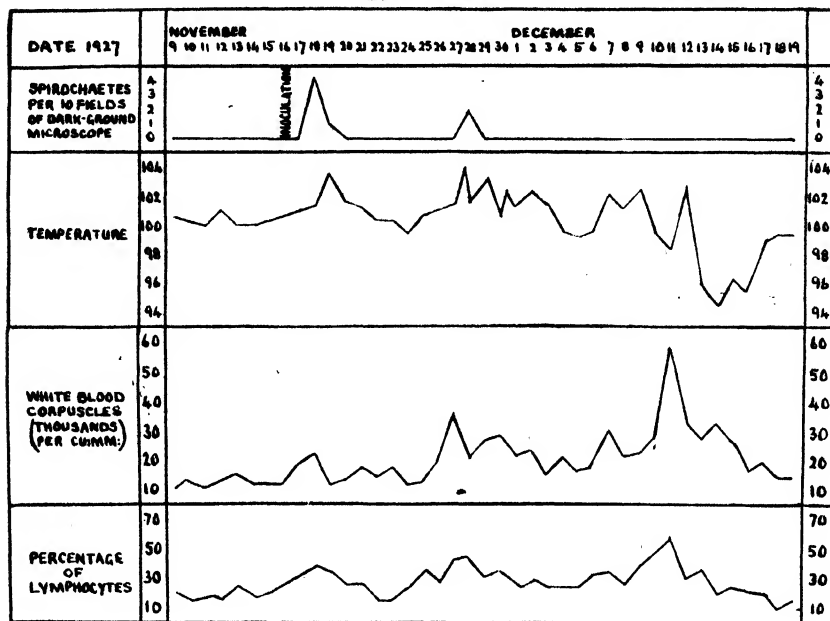
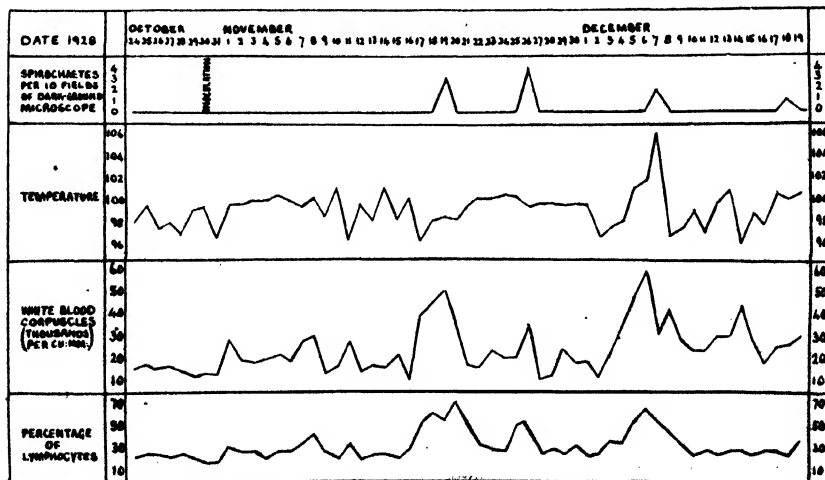


CHART 2. — FEMALE MONKEY — INOCULATED SUBCUTANEOUSLY WITH TREPONEMA DUTTONI, 30th OCTOBER, 1928



NOTE.—Magnification of dark-ground microscope = 720.

relative lymphocytosis during the intervals is seen only when a relapse is to occur. A leucocytosis during the attacks, due to an increase in the number of polymorpho-nuclear neutrophil cells, has been noted by many observers, e.g., Suldey, Mayer, Kartacheff (1925) and Höglund (1927), and Karawacki and Krakowska state that this leucocytosis persists for some days after the crisis. It is interesting to note that Manson and Thornton (1919) found a leucocytosis in the cases in East Africa only when the temperature was high or when some complication such as severe bronchitis was present. Kartacheff's observations of phagocytosis of the siprochaetes and the deflection of the Arneth Index to the left were not confirmed. According to Mayer this change in the Arneth Index persists if the spirochaetes remain in the blood after the febrile period.

Several workers have noted during the febrile stages either entire absence or unusual paucity of the *eosinophil cells* (Jouveau-Dubreuil, 1919; Lebouef and Gambier, 1919; and Suldey). The first-mentioned points out that in his cases in Western China this aneosinophilia, which incidentally he found to be the only marked alteration from the normal blood, is especially noteworthy owing to the prevalence of concomitant parasitic infections of the intestine. He also states that the number of eosinophils does not rise in the intervals until after the final attack. Suldey's contention, however, that in the intervals there may be a relative eosinophilia throws doubt on the efficacy of using this sudden rise, as suggested by Jouveau-Dubreuil, as an aid in determining, after the fever has fallen, whether or not the disease has come to an end.

It is worthy of note that D'Avigny working in the Soudan epidemic of 1923 obtained results very different from the above. He asserts that the number of polymorph cells which was slightly below normal at the commencement of the disease, showed a progressive diminution up to the crisis, after which there was an increase which reacted its maximum just before the relapse. This discrepancy may, to some extent, be due to the fact that D'Avigny was working with Singalese troops, for Van den Branden and Van Hoof (1922) in the Congo allege that the leucocyte formula in natives is naturally very irregular.

IMMUNITY AND SUPER-IMPOSED INFECTION.

It was found impossible to re-infect mice or rats which had recovered from the disease for varying periods up to eight months—a result in keeping with the observations of Weichbrodt (1921), Sagel (1928) and Bruynoghe and Dubois (1928). Kroó (1925) too, found that animals recovered from an infection with a Russian strain were immune to the same strain until after the latter's passage through a clean *Ornithodoros moubata* when re-infection became possible. Nevertheless, by some the immunity is not considered to be absolute, for Todd (1922) states that a patient may suffer from the disease twice in a single winter, and Margolis (1919), Selwyn-Clarke, Le Fanu and Ingram (1923), Nicolle and Conseil (1923), Werner (1924), Oliver (1924), J. L. Kritschewsky and Brussin (1926) and Plaut (1928b), have all recorded what they believe to be re-infections at varying intervals after recovery either in the human subject or in animals. Hermonius (1928), too, by the administration of very large doses believes that he was able to re-infect mice which had recovered from an attack caused by *Treponema duttoni* five or six weeks previously. Weichbrodt (1920), working with the Hamburg and Elberfeld strains of the same organism, found that mice developed an immunity which was specific only for the homologous strain, and Bruynoghe and Dubois (1928) have suggested that the diversity of opinions on the subject of immunity probably results from the use of strains not strictly homologous.

SUPER-IMPOSED INFECTION. In determining that a reappearance of the spirochaetes in the blood following a second inoculation is due to a re-infection and not to a provocation of one still persisting, J. L. Kritschewsky and Brussin (1926) emphasise the difficulty in determining whether an animal has become entirely free from the organisms which caused the original infection from which the animal has apparently recovered. The discovery by Buschke and Kroó of latent forms in the brains of such animals apparently recovered renders this difficulty peculiarly acute. The writer, however, has produced what he believes to be *an infection super-imposed on a mild primary infection* comparable to those recorded by Kudicke, Feldt and Collier (1924), Steiner and Steinfeld (1926), J. L. Kritschewski and Brussin (1926) and Prigge (1926b and 1926c). Six mice were given simultaneously small equal doses of the same

infected blood. Four days later three of these mice received a further and much heavier dose, and all—as judged by the number of spirochaetes observed in the blood and by the unusual length of the attack—became much more severely infected than the three animals each of which had received only one dose (see Table II). Similar results were obtained with *Spirillum minus*, and the writer, believes that he himself suffered from a super-imposed infection (Gray, 1928). It is interesting to note, too, that Strempel and Armuzzi (1927a) found that mice inoculated intraperitoneally became immune to further intraperitoneal inoculation, but were often susceptible to super-imposed infection by the subcutaneous route.

SERUM.

In vitro experiments have been described in a previous paper (Gray, 1928). In contradistinction to Sawtschenko and Melkich (1901) and Novy and Knapp (1906), the presence of antibodies was not demonstrated in either the writer's own serum withdrawn when he was convalescent; or in the sera of various animals taken at a crisis and during attacks, intervals and convalescence. No agglutination or lysis was noted even after incubation at 37°C. for twenty-four hours. It was considered necessary to test the sera with at least three generations of spirochaetes (in different animals) in view of the work of Levaditi and Roché (1907), Janszó (1918), Kudicke and Feldt (1924), Cunningham (1925), Brussin (1925), Brussin and Rogowa (1927), Gori (1928) and Meleney (1928), who observed immunological differences between the spirochaetes of the first attack and of the relapses. Failures to demonstrate antibodies in the serum of convalescents have been recorded by Bruynoghe, De Greef and Dubois (1927), and by Plaut (1928b), even although the latter proved the patients to be completely immune to subsequent inoculation. Passage through laboratory animals, too, evidently tends to obliterate any serological distinctions originally demonstrable such as differences in antigenic properties (Kudicke, Feldt and Collier, 1924) or differences elicited by Rieckenberg's adhesion test and immunity reactions (Brussin and Schapiro, 1928). Kroó (1925), however, managed by passage through a 'clean' *Ornithodoros moubata* to transform a Russian strain which had been maintained in mice into a distinct serological strain.

An attempt made to distinguish between various generations of relapsing fever spirochaetes by means of Rieckenberg's reaction was unsuccessful, although occasionally the adhesion phenomenon was observed with platelets and bacteria when spirochaetes were brought into contact with the serum of convalescent animals. Brussin (1925 and 1926), however, states that in relapsing fever the thrombocyto-barin on which the reaction depends, develops one or two days after the natural or therapeutically-induced crises in mice, and he has thereby been able to differentiate strains of the first and second attacks. Later, in 1927, in conjunction with Rogowa he showed that in mice inoculated with organisms of the second, third or fourth attacks, the spirochaetes of the first attack were serologically distinct from those of the inoculum, but the organisms of the first relapse were identical. Krantz (1926b) showed that under the action of the specific serum or plasma, platelets and bacteria adhered to the spirochaetes only when the latter were alive.

The results with the writer's serum and with certain of the animal sera withdrawn after recovery may have been due to the diminution, during the convalescence, of antibodies originally in the blood for Löwenthal, who has used an agglutination reaction for diagnostic purposes when spirochaetes cannot be demonstrated, found that the agglutinating power of both human and animal blood was greatest during the crisis of the attack and after the administration of spirochaetical drugs. Wenyon (1926), too, considers it probable that 'antibodies which bring about the disappearance of the spirochaetes in the blood, do not persist long enough to prevent relapse.' Nevertheless, several of the specimens of blood tested were withdrawn from a mouse or rat at the time of a crisis and even these failed to produce any noticeable effect on successive generations of spirochaetes *in vitro*. This would suggest that immunity against re-infection does not depend upon the presence of immune substances in the blood.

In vivo experiments were also performed. Attempts were made to protect and cure mice with (1) 'immune' sera, (2) 'hyper-immune' sera, and (3) 'vaccines.'

(1) '*Immune*' sera. Three doses, each of 0.75 c.c. of the writer's blood withdrawn when he was convalescent, and inoculated subcutaneously into mice at intervals of from three to four days, not only failed to protect them from infection but, contrary to the results of

Brienl and Kinghorn (1906), did not even prolong the incubation periods. As with the *in vitro* experiments these results probably were not due to the diminution during convalescence of any protective substance which may have at one time been present, for blood withdrawn from a rat or mouse at a crisis failed to protect or cure mice from infection with the spirochaetes of various successive attacks. It is possible, of course, that the amounts of serum used were too small, for Sagel (1928) found that convalescent serum, to be of any use, had to be exhibited in very large doses.

(2) '*Hyper-immune*' sera (i.e., sera derived from rats after a varying number of inoculations with infected blood) were tried for both the prophylaxis and treatment of the disease in mice, but neither was the incubation period prolonged nor the severity of the attack moderated as recorded by Breinl and Kinghorn (1906), nor were there attained the very favourable results obtained by Novy and Knapp (1906) in the treatment of infections once established.

(3) '*Vaccines*.' Samples of defibrinated rats' blood very rich in spirochaetes were kept for forty days at 0°C. and at room temperature and then inoculated into healthy mice. Unlike the results of Novy and Knapp it was found that blood so treated, even when taken early in the attack was not infective and even when obtained in the later stages did not confer immunity to subsequent inoculation with material known to be infected. The inefficacy of killed spirochaetes to produce immunity has been demonstrated by Reiter (1925) and Bruynoghe (1928) and Krantz (1926b), by means of the Rieckenberg reaction, demonstrated the presence of an anti-body which is not developed by the injection of dead spirochaetes but only after actual infection.

In experiments to find the influence of dosage on the incubation period a number of mice were given a series of graded doses of infected mouse-blood. Several of the mice which received exceedingly high dilutions of the blood in citrate solution did not become infected but, on being tested later, were all found to be still susceptible. Reiter (1925), however; by injecting decreasing doses of cultural forms into a series of mice arrived at a dose which did not produce any evident infection and yet caused an immunity comparable to that which succeeds an evident infection.

Experiments practically identical with those of Gori (1928) and

similar to those of Jansc6 (1918), were also performed in an endeavour to distinguish by immunity reactions, the spirochaetes of the various successive attacks in any one mouse. Sub-inoculations were made during the first, second and third attacks of a mouse into further mice which, after recovery from the resulting infection, were found to be completely immune not only to the spirochaetes of the original attacks in each case as in Gori's experiments but also to those of the other stages of the disease.

All experiments performed in an attempt to demonstrate *Pfeiffer's reaction* in mice with this strain of *Treponema duttoni* failed. Two series of experiments were carried out. (1) Actively-motile spirochaetes contained in mouse-blood were introduced into the peritoneal cavities of a series of mice which had become immune through recovery from infection and, as a control experiment, a series of healthy non-immune mice. At periods of one, two and four hours, and then daily for six days subsequent to the inoculations, fluid from each of the peritoneal cavities was removed by a pipette and examined by the dark-ground illumination method. In no case was there noted any difference between the two series of animals except, of course, that the non-immune mice developed the disease as shown by the presence of the spirochaetes in the blood, while the others did not become infected.

(2) Actively-motile spirochaetes contained in mouse-blood were introduced into the peritoneal cavities of normal mice, some along with $\frac{1}{2}$ c.c. serum of another mouse obtained at the crisis or obtained after recovery from all attacks, and others with $\frac{1}{2}$ c.c. of the writer's serum taken subsequent to his recovery from an accidental infection. Control experiments in which no serum was introduced, were performed in parallel. As before, successive samples of the peritoneal fluid were examined by the dark-ground illumination method. No difference was noted, however, between the two series of mice, all the mice of both series becoming infected in this case.

PERSISTENCE OF SPIROCHAETES IN THE TISSUES.

Spirochaetes may persist in the body after the apparent recovery of the host. Healthy mice were inoculated with the heart-blood and emulsions of various organs of recovered animals. By this method latent forms were found in the *brain* of a mouse which had

experienced its last attack thirty-eight days prior to being killed, even although its heart-blood and other organs were negative. This is in keeping with the work of Kroó (1926) and of Schauder (1928) but contradictory to the assertion of Prigge (1926b and 1926c) that if spirochaetes are present in the brain they must also necessarily be present in the circulating blood. Latent forms in the brain were first demonstrated by Buschke and Kroó (1923), the latter worker showing in 1926 that the brain may harbour them as long as ninety days after apparent recovery. Indeed, it has been suggested that immunity to further infection lasts only for, and is due to the persistence of the spirochaetes in the brain (Bruynoghe, 1928), but this has been contradicted by Prigge (1926b and 1926c). Steiner and Steinfeld (1925), and Stempel and Armuzzi (1927a), believe that they are not resistant to antibodies in the serum of the same animal. These persistent forms in mice brain are said to be more commonly found after re-infections (Hermonius, 1928), and when the strain of infecting spirochaete has recently been isolated from a human case, than when the organism has been maintained for long in laboratory animals (Prigge and Rothermundt, 1928).

Steiner and Schauder (1925) have demonstrated persistent forms in the bone marrow as well as in the brain, and believe, in contradistinction to Stempel and Armuzzi (1927a), that the presence of resistant forms is a regular phenomenon. Levaditi and Anderson (1928) believe the persistent forms in the brain, as they are invisible yet not filterable, to be intimately associated with the nerve cells, and Plaut (1928a) has shown that splenectomy has no effect on them.

Although several workers, including Krantz (1926), Kroó (1926), Johannessohn (1926), Lebedjeva and Ssinjuschiva (1927) and Schauder (1928), have demonstrated these latent forms even after treatment with salvarsan, Johannessohn states that they are not salvarsan-fast, and Prigge (1926b and 1926c) and Schreus and Weisbecker (1926) assert that when sufficient and early doses of salvarsan are given, the spirochaetes quickly disappear from the brain of mice.

J. L. Kritschewski (1927) found that different races of relapsing-fever spirochaetes differ in their power to persist in the central nervous system and claimed to have shown the existence of neurop-tropic as distinct from somatotropic races of spirochaetes. Steiner

and Steinfeld (1927) and Plaut (1928a) believe that these latent forms never re-invade the blood-stream 'which, with its antibodies appears to act as an impenetrable barrier.' Aznar (1926), however, showed that the injection of *Trypanosoma brucei* or *Trypanosoma gambiense* into a mouse, recovered from a spirochaetal infection five months previously, would cause a reappearance of the spirochaetes in the blood two or three days later, and that the spirochaetes sometimes persisted there one or two days after the trypanosomes had appeared in the blood. Savini (1923), Wenyon (1927), Vincent (1927) and Bruynoghe (1928), have obtained similar results and the writer has found that inoculation with *Trypanosoma brucei* of rats and mice apparently recovered from infections with *Treponema duttoni* and *Spirillum minus* may cause a reappearance of the spirochaetes in the circulating blood in from one to four days. In over twenty mice which had recovered from infection with *Treponema duttoni* from periods up to four and a half months previous to the inoculation with the trypanosomes it was found that if the last attack had occurred more than one month before, no reappearance of the spirochaetes was produced. In addition, the observation of Bruynoghe (1928) was confirmed in that infection with *Spirillum minus* of a mouse recovered from relapsing fever two or three months previously will often cause the spirochaetes to reappear.

It is noteworthy that Joseph (1926) showed that in rats which had apparently recovered from a relapsing fever infection, inoculation with nagana trypanosomes did not clear the brain of the spirochaetes.

• TREATMENT WITH ORGANIC ARSENICALS AND BISMUTH.

The preparation of arsenic which the writer used first was 'sulphostab,' i.e., 'diaoxydiamino-arsenobenzol-sodium formaldehyde-bisulphite,' as prepared by Messrs. Boots Pure Drug Co. Ltd. This preparation was chosen as being one which could be administered subcutaneously without pain or subsequent local reaction. Doses of from 0.04 to 0.32 grams per kilo. of body weight given at intervals of six to seven days were found to be valueless in protecting and treating mice.

TABLE III.

DEMONSTRATING THE ABSENCE OF PROTECTIVE PROPERTIES OF THERAPEUTIC DOSES OF 'SULPHOSTAB' FOR MICE.

Each of the mice 206 to 211 was given subcutaneously a therapeutic dose of the drug (in the proportion of 0.04 gram to each kilo of body weight) every 6th or 7th day, and was inoculated with infected blood on the 3rd day after the first dose. Mouse 211A was used as a control, being given no 'sulphostab.' Day 1 denotes the day on which the animals were inoculated with the infected material. (The figures are used as in the previous Tables.)

Day			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Mouse 206	0	0	0	0	0	1	2	0	0	1	0	1	1	1	D
" 207	0	0	0	0	1	1	3	0	1	2	0	1	1	1	D
" 208	0	0	0	0	0	0	2	2	0	1	1	1	1	0	0
" 209	0	0	0	0	0	0	2	1	0	1	0	0	0	0	0
" 210	0	0	0	0	0	1	2	5	1	2	0	1	0	0	1
" 211	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0
" 211A	0	0	0	0	1	3	5	1	0	1	0	0	0	0	1

TABLE IV.

DEMONSTRATING THE ABSENCE OF PROTECTIVE PROPERTIES OF HYPERTOXIC DOSES OF 'SULPHOSTAB' FOR MICE.

Each of the mice 212 to 215 was given subcutaneously a varying dose of the drug every 6th or 7th day, and was inoculated with the infected material at the same time as the first injection of the drug. Mouse 220 was used as a control, being given no 'sulphostab.' (The figures are used as in the previous Tables.)

			Number of Therapeutic doses given every 6 or 7 days	Days														
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Mouse 212	1	0	0	0	0	1	2	4	1	1	0	1	1	3	0	0
" 213	2	0	0	0	0	1	1	3	2	1	0	0	0	0	1	1
" 214	4	0	0	0	0	0	1	2	5	0	0	1	0	0	1	2
" 215	8	0	0	0	0	0	1	1	4	1	1	1	0	2	1	1
" 220	0	0	0	0	0	0	1	3	5	1	0	1	0	0	0	0

'Bismostab,' a suspension of finely-divided metallic bismuth in 5 per cent. glucose solution also prepared by Messrs Boots, was administered subcutaneously in doses equivalent by weight to that recommended as the therapeutic dose for man and up to eight times that amount, but was also found to be valueless for prophylaxis.

TABLE V.

DEMONSTRATING THE ABSENCE OF PROTECTIVE PROPERTIES OF THERAPEUTIC AND HYPERTOXIC DOSES OF 'BISMOSTAB' GIVEN EVERY 6TH DAY, THE FIRST DOSE BEING GIVEN SIMULTANEOUSLY WITH THE INOCULATION WITH THE INFECTED MATERIAL

A therapeutic dose of the drug was calculated as 3.6 gm. per kilo. of body weight. Mouse 225 was used as a control, being given no 'bismostab.' (The figures are used as in the previous Tables.)

	Number of Therapeutic doses of 'bismostab' given every 6th day	Days															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Mouse 221 . . .	1	0	0	0	0	0	0	4	1	0	1	1	0	2	0	1	2
„ 222 . . .	2	0	0	0	0	0	0	3	1	1	0	0	0	1	1	0	0
„ 223 . . .	4	0	0	0	0	0	1	4	1	0	0	1	1	1	0	0	0
„ 224 . . .	8	0	0	0	0	0	0	3	1	1	0	0	0	0	1	1	0
„ 225 ...	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	1	0

'Sulphostab,' in conjunction with 'bismostab' was then tried. While amounts equal to eight times the ordinary therapeutic doses of the two drugs when given together were in one case found to protect, smaller amounts were of no effect, and doses equivalent by weight to ten times the recommended amounts for man were rapidly fatal. The margin between the efficient and the lethal doses of the two drugs was, therefore, of the smallest.

TABLE VI.

DEMONSTRATING THE PROTECTIVE PROPERTIES OF HYPERTOXIC DOSES OF 'SULPHOSTAB' AND 'BISMOSTAB' GIVEN SIMULTANEOUSLY EVERY 6TH DAY

Mice 226 to 229 were given varying doses of the drugs, the first doses being administered simultaneously with the inoculation with the infected material. Mouse 229, which received amounts equal to 8 times the ordinary therapeutic doses, did not develop the disease, but mice 226 to 228, which all received smaller doses, and the control mouse 230, which received no drug at all, all became infected. A therapeutic dose of 'sulphostab' was calculated as 0.04 gram, and of 'bismostab' as 3.6 gram per kilo of body weight. (The figures are used as in the previous Tables.)

	Number of Therapeutic doses given every 6 days		Days															
	'Sulpho- stab'	'Bismo- stab'	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Mouse 226 . . .	1	1	0	0	0	0	0	1	2	5	1	1	2	1	3	3	1	0
„ 227 ..	2	2	0	0	0	0	0	0	0	4	2	1	0	1	0	0	0	0
„ 228 ..	4	4	0	0	0	0	0	2	1	2	1	0	0	1	2	1	1	0
„ 229 . .	8	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
„ 230 ..	0	0	0	0	0	0	0	0	1	3	1	1	1	0	0	0	1	0

Sulfarsenol was then administered to rats intramuscularly and '914' intravenously in doses of from one to four centigrams per kilo. of body weight. Apart from the fact that the incubation period was increased with these drugs on the average by two days beyond the five days of the control rats, there was no noticeable effect of the drug.

In view of the high resistance of the strain to arsenic as shown by the above experiments with *sulfarsenol*, '914' and also by the inefficacy of intravenous injections of *novarsenobillon* in the case of the writer who himself became accidentally infected, any deprecatory remark as to the therapeutic efficiency of the drugs tried would be unwarranted. Numerous infections with arsenic resistant strains are recorded both in humans (for references see Gray (1928)) and in animals (J. L. Kritschewsky and Ljass (1925), Collier (1925), Plaut (1925), L. W. Kritschewsky (1926), Bruynoghe (1928), and P. Nicolle (1928)). J. L. Kritschewsky (1927b) found considerable variations in the susceptibility of seven different strains of relapsing-fever spirochaetes to therapeutic doses of salvarsan and Krantz (1926a), has brought forward some evidence suggesting that the action of salvarsan is correlated with the development of antibodies so that it is not necessarily greatest in the early stages of the infection.

SUMMARY

Various experiments on laboratory animals with a strain of *Treponema duttoni* are recorded, and the results obtained are compared with those of other workers.

Susceptible Animals. Mice, rats, a young guinea-pig and *Macaci sinici* were successfully infected. Adult guinea-pigs and rabbits proved refractory.

Avenues of Infection. Inoculation of susceptible animals with infective material by the subcutaneous and intraperitoneal routes always produced the infection. It was found that the spirochaete could pass through the unbroken skin and mucuous membranes of the nose and alimentary canal. The question of placental transmission is discussed.

The *incubation period* for mice varied between one and sixteen days. Factors influencing the length of the period have been studied.

The Attacks. Several attacks were experienced by mice, rats and monkeys, the greatest number of these species respectively being five, three and four. The duration, severity and mortality of the attacks have been studied.

The Spirochaetes. The duration of the presence, the numbers and the distribution of the organisms in the blood have been studied. Frequently, about the middle of a prolonged first attack in a mouse, there was observed a marked decrease in the number of organisms present in the circulating blood.

The Cellular Changes in the Blood, as observed in the monkeys, took the form of (1) a progressive diminution in the number of red cells and in the haemoglobin associated with the presence of reticulocytes, and (2) during each attack a leucocytosis with a marked relative lymphocytosis.

Immunity and Serology. Mice and rats could not be re-infected, but a second infection could be super-imposed on an existing primary one.

Antibodies were not demonstrated in the blood of an infected human subject and animals either by *in vitro* or *in vivo* experiments even when the serum was obtained at a crisis. No protective or curative properties could be ascribed to 'immune' sera, 'hyper-immune' sera or 'vaccines.' The spirochaetes of the various attacks in any one animal could not be distinguished by immunity reactions, and efforts to demonstrate the Pfeiffer Reaction failed.

Persistent Forms. Persistent forms were demonstrated in the brains of mice after apparent recovery even when the blood was not infective to susceptible animals.

Inoculation of an apparently recovered mouse or rat with *Trypanosoma brucei* or *Spirillum minus* sometimes caused the spirochaete to reappear in the circulating blood after it had disappeared following recovery.

Arsenic Resistance. The strain was markedly resistant to organic arsenicals. Arsenical preparations, either alone or combined with bismuth, had neither prophylactic nor therapeutic value except in one mouse to which was administered hypertoxic and almost lethal doses of 'sulphostab' combined with 'bismostab.' The only noticeable effect of the intramuscular administration of sulfarsenol and intravenous injection of '914' to rats was slightly to lengthen the incubation period.

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THE DISTRIBUTION OF SANDFLIES AND LEISHMANIASIS IN PALESTINE, SYRIA AND MESOPOTAMIA

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PLATE IV AND TWO MAPS

From May 12th, 1928, to October 20th, 1928, we carried out a survey of sandflies in Mesopotamia, Syria and Transjordan, in relation to the distribution of Leishmaniasis in addition to the study of sandflies in Palestine on which we have been engaged since the summer of 1924. We concentrated particularly on localities from which Leishmaniasis has been recorded but we also examined districts in which Leishmaniasis is unknown. Obviously a survey of such a large area in the space of one summer cannot be complete, but it indicates the salient features in the distribution of the more important species, for it includes the extremes and all gradations of topographical and climatic variations within the large area examined.

In carrying out this work we were greatly helped by the Public Health authorities and we have to thank Dr. Briercliffe of Jerusalem, Dr. Blofield of Amman, Major Hallinan, Major Hegg, Dr. A. E. Mills, Dr. Hannah Khayat and Dr. Sammi Beg of Baghdad, Colonel Causeret and Dr. Mandour of Beyrout, Colonel Dagorn and Major Sondag of Aleppo.

The distribution of Leishmaniasis in the countries under consideration is probably wider than the literature on the subject indicates, for with the increasing use of microscopical and cultural methods of diagnosis new foci are being discovered.

Cutaneous Leishmaniasis apart from the large endemic centres in Baghdad, Mosul and Aleppo is distributed irregularly throughout the whole area examined.

Visceral Leishmaniasis has been recorded only from the Lebanon. Lépine and Hitti (1927) recorded three cases, Professor Vregille of the French University of Beyrout has observed two cases of infantile Leishmaniasis in Beyrout City and Professor Hinshaw, of the American University of Beyrout, informs us that he observed one case from Tripoli.

Cutaneous Leishmaniasis in dogs is known to occur in Baghdad and Aleppo. Visceral canine Leishmaniasis has not yet been recorded. Buxton (1923) who examined 156 dogs in Jerusalem, Wenyon (1911) who examined 110 dogs in Baghdad, and Chadwick and MacHattie (1927) who examined 130 dogs in Baghdad did not find evidence of visceral Leishmaniasis.

METHOD OF WORK

Sandflies were dissected in saline and the alimentary tract examined for Leishmania. The diagnosis was based in the case of females on the spermathecae, pharynx and buccal cavity, in the case of males on the external genitalia, pharynx and buccal cavity. In addition preserved material was mounted and examined, care being taken to mount every head in a horizontal position. The above mentioned characters are the only ones which are legitimate for the diagnosis of specimens of the genus *Phlebotomus* for other characters such as wing venation, palp and antennal formula are subject to great variation and when used alone give rise to confusion. Size and colour are also of no value for diagnosis for they vary enormously in each species.

DIAGNOSIS OF SPECIES FOUND

The character of the male genitalia is insufficient for specific diagnosis, e.g., in *P. major* and *P. chinensis* the male genitalia are similar and the pattern of the male genitalia is the same throughout the whole minutus group. It is probable that the male genitalia will eventually prove to be of generic value. In our opinion sandflies cannot be considered as belonging to one genus but should be raised to the rank of a family, *Phlebotomidae*. For the purpose of this paper we refer all sandflies to the genus *Phlebotomus*, for a revision is not yet possible since most of the species are insufficiently described.

Phlebotomus papatasi Scop.

The pharynx of this species (Plate IV, fig. 1) has been described (1926) and the spermathecae (fig. 3, *a*) have been described and figured by Grassi (1907) and Newstead (1911). This is the most widely distributed and commonest species in the area examined.

Distribution and material examined.

PALESTINE AND TRANSJORDANIA :—Jerusalem : several thousand. Jericho : several thousand. Haifa : 250. Amman : 120. Djerash : 60. Maan : 70. Mozah : 300. Rosh Pinah : 300. Tiberias : 120. (Males and females.)

SYRIA :—Aleppo : 570 ♀♀ (no males caught). Hellaweh : 2 ♂♂, 1 ♀. Tripoli : 6 ♂♂, 4 ♀♀. Batroun : 8 ♂♂, 4 ♀♀. Kubbah : 8 ♂♂, 10 ♀♀. Enfe : 19 ♂♂, 16 ♀♀. Beyrout : 6 ♀♀, 2 ♂♂. Zachle : 3 ♂♂, 7 ♀♀. Adde : 13 ♂♂, 2 ♀♀. Bcherreh : 1 ♀. Bet Meri : 1 ♂, 3 ♀♀. Bar Elias : 36 ♂♂, 43 ♀♀.

MESOPOTAMIA : Baghdad : 528 ♀♀, *circ.* 100 ♂♂. Mosul : 40 ♀♀, 6 ♂♂. Basrah : 29 ♀♀.

Phlebotomus sergenti Parrot (1917).

SYNONYMS :—*P. sergenti* var. Newstead (1920).

P. sergenti var. *alexandri* Sinton (1928).

This species was first recorded from Algeria by Parrot. This author described only the male for at that time (1917) no criteria existed for diagnosing females of the genus *Phlebotomus*. Later this species was recorded also from other localities. Sinton (1925) described the distribution of this species.

DESCRIPTION

FEMALE.

Size : 1.6 mm. to 3 mm.

Palp formula : 1, 2, 4, 3, 5 or 1, 4, 2, 3, 5 or 1, (2, 4), 3, 5. Segment 5 is about as long or slightly larger than segment 2 + 3.

Antennae : Geniculated spines on segments 3 to 15. Segment 3 > 4 + 5. In one specimen from Bcherreh : 3 < 4 + 5.

Pharynx (Plate IV, fig. 2; and fig. 2, *b*) : The dentition is very characteristic and at once distinguishes *P. sergenti* from *P. papatasi*, *P. major* and *P. chinensis*. Posteriorly for a short distance the

teeth of the dorsal plate appear in optical section as long curved scales. Anteriorly they appear as long curved teeth with their axis longitudinal or slightly oblique. The real shape of the teeth in the pharynx is very difficult to determine in mounted specimens viewed dorsally. Their character can only be made out with certainty in sagittal sections. It should therefore be understood that the descriptions given in this paper refer only to the dorsal view of a flat mounted head.

The above features were noted in one specimen from Algeria presented by Dr. L. Parrot, of the Institut Pasteur d'Algérie, in specimens from North China presented by Professor Patton and Dr. Young, and in material caught in Mesopotamia and Syria.

The buccal cavity contains no armature.

Spermathecae (fig. 3, c): They consist of five or six segments (never less than five). The superior segment is rounded and larger than the others which diminish in diameter from before backwards. The duct widens considerably at its junction with the body of the spermathecae. The above type of spermatheca was found in specimens caught in Mesopotamia and Syria.

MALE.

Size: 1.5 mm. to 3 mm.

Palps and *Antennae* as in the female.

Pharynx (Plate IV, fig. 3; and fig. 2, a): As in many sandflies the pharynx of the male is different from that of the female. It is narrower and the teeth of the dorsal plate are much less developed than in the female, sometimes being reduced so much that they appear as a network of lines as in the female of *P. papatasi*. The teeth of the lateral plates extend very far anteriorly. They point backwards and appear very conspicuous on account of the feeble development of the teeth on the dorsal plate. This type of pharynx was seen in males from Algeria, Northern China, Mesopotamia, Syria and in two males from the Caucasus presented by Professor Pavlowsky. (These two specimens are probably *P. li* Popow. They will be referred to later.)

Newstead (1920) described *Phlebotomus sergenti* var. on the basis of two males caught in Amara. Newstead created this variety on the following grounds; the third segment of the antennae is relatively much shorter than in *P. sergenti* and the distal spine bearing

processes of the second segment of the superior clasper are markedly unequal in length, the terminal process being three times as long as the other in contrast to *P. sergenti*, in which the processes are equal. The difference in the antennae noted by Newstead is unimportant as the relative length of these organs and their segments is subject to variations. Newstead's figures when measured show the same relation between the third and fourth segment both in *P. sergenti* var. and *P. sergenti* Parrot. Newstead illustrates the difference between the genitalia of *P. sergenti* var. and *P. sergenti* in a diagram. The figure given by Newstead for the genitalia of *P. sergenti* var. is exactly similar to that given by Parrot (1917) in his original description of *P. sergenti*. The apparent differences noted by Newstead depend purely on the position of the genitalia in mounting. We had an opportunity of studying this point in a large number of males caught in Baghdad. The terminal spine bearing processes of the distal segment of the superior claspers are almost constantly equal but mounted laterally one often appears longer than the other. Sinton (1928) accepting Newstead's description of *P. sergenti* var. created the variety of *P. sergenti* var. *alexandri*; for reasons given above we consider this a synonym of *P. sergenti* Parrot.

P. sergenti differs from *P. sergenti* var. *mongolensis* Sinton (1928) in the following points. In the female the spermathecae of *P. sergenti* var. *mongolensis* consist of only four to five segments (fig. 3, *d*); as in *P. sergenti* the superior segment is much wider than the others. The superior clasper of *P. sergenti* is about two and a half times as long as it is broad; in *P. sergenti* var. *mongolensis* it is five times as long as broad. The distal spine bearing processes of *P. sergenti* when seen from above are equal in length; those of *P. sergenti* var. *mongolensis* are markedly unequal (fig. 1, *c-d*). The intromittant organ in *P. sergenti* tapers uniformly, ending in a little knob, sometimes a rudimentary hook; in *P. sergenti* var. *mongolensis* it is much blunter and the dorsal hook is rather strongly developed. The intromittant organ of *P. li* is very similar to that of *P. sergenti* var. *mongolensis* (fig. 4, *d-e*). In all other respects *P. sergenti* and *P. sergenti* var. *mongolensis* are indistinguishable.

We compared *P. sergenti* from Algeria, Mesopotamia and Syria, with two specimens from the Caucasus presented by Professor Pavlowsky. The only difference noted was that the pedicle bearing a brush on the

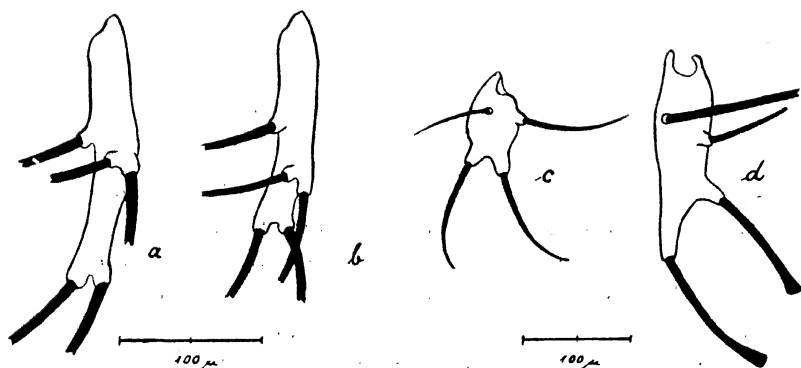


FIG. 1. Second segment of superior clasper of male of (a) *Phlebotomus major*; (b) *Phlebotomus major* var. *perniciosus*; (c) *Phlebotomus sergenti*; (d) *Phlebotomus sergenti* var. *mongolensis*. (c) and (d) ventral view.

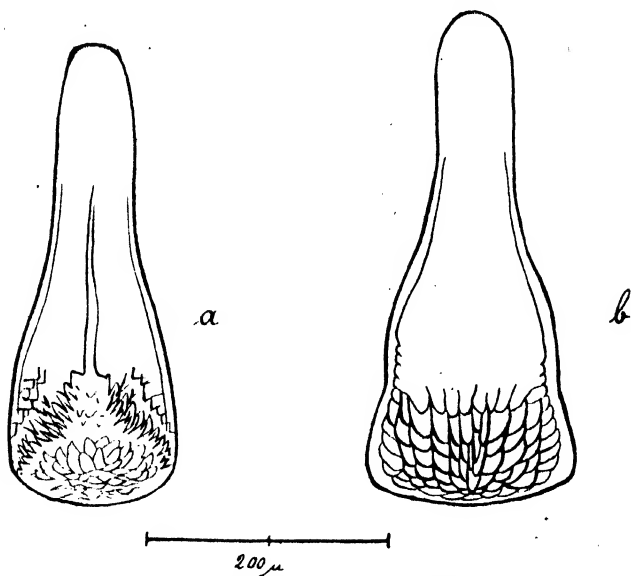


FIG. 2. Pharynx of *Phlebotomus sergenti*. (a) Male; (b) Female.

first segment of the superior clasper was much wider than that of *P. sergenti*. The broad pedicle is a constant feature for *P. li* Popow (1925), and Sinton (1928) did not find intermediate forms between *P. li* and *P. sergenti*. Until females of *P. li* are examined it will be impossible to decide whether *P. li* is a distinct species or, as Parrot thinks, a variety of *P. sergenti*.

Distribution and material examined. Baghdad: 683 ♀♀, 200 ♂♂. Mosul: 22 ♀♀. Aleppo: 111 ♀♀, 50 ♂♂. Bcherreh: 1 ♀.

Phlebotomus major, Annandale (1911).

The diagnostic characters in this species are to be found in the spermathecae and the pharynx in the female, pharynx and external genitalia in the male.

Pharynx (Plate IV, fig. 4): The teeth appear in both sexes as parallel rows of points. The lateral part of the toothed area extends anteriorly as far as or slightly further than the median part.

Spermatheca (fig. 3, b): The spermathecae consist of about twelve segments and have a long thin tubular process (neck according to Sinton) terminating in a little club-shaped thickening bearing a tuft of hairs.

Male genitalia. The male genitalia in general outline resemble those of *P. chinensis* but, as Sinton (1928) has pointed out, the intromittant organ in *P. major* has a blunt rounded tip, while in *P. chinensis* it bears a tubercle on its ventral side near the tip (fig. 4, b).

França and Parrot (1921) considered *P. perniciosus* Newstead to be a variety of *P. major* and named it *P. major* var. *perniciosus*. Newstead (1911) in his original description of *P. perniciosus* pointed out that the intromittant organ is bifid (fig. 4, c). An examination of specimens from Algeria and France showed all the males to have a bifid intromittant organ, i.e., they are *P. major* var. *perniciosus*. The pharynx in both sexes and the spermathecae in the females are similar to those of *P. major*. In *P. major* var. *perniciosus* the two middle spines on the superior clasper are situated half way between the proximal one and the two terminal ones; in *P. major* from Palestine and Syria the two middle spines are always situated very near the proximal spine (fig. 1, a-b).

In Syria and Palestine we found only males with an intromittant

organ characteristic of *P. major*. We therefore assume that *P. major* var. *pernicius* is absent or very rare in Syria and Palestine.

Distribution and material examined. Jerusalem : 5 ♀♀, 3 ♂♂. Mozah : 15 ♀♀, 6 ♂♂. Haifa : 30 ♀♀, 12 ♂♂. Rosh Pinah : 9 ♀♀, 4 ♂♂. Aleppo : 1 ♀. Batroun : 4 ♂♂, 2 ♀♀. Kubbah : 5 ♂♂, 2 ♀♀. Enfeh : 1 ♂. Bcherreh : 1 ♂, 8 ♀♀. Cedar Grove : 1 ♀. Beth Meri : 4 ♂♂, 11 ♀♀. Bar Elias : 2 ♀♀. Adde : 7 ♂♂, 5 ♀♀. Zachle : 2 ♀♀.

Phlebotomus chinensis Newstead.

SYNONYM :—*P. major* var. *chinensis* Newstead (1916).

This species has until recently been considered a variety of *P. major*. The authors (1928) pointed out that *P. chinensis* must be considered a distinct species for the pharynx and spermathecae in the female and the pharynx in the male are very different from those of *P. major*. Sinton (1928) and Sinton and Barraud (1928) have described and figured the essential differences between *P. chinensis* and *P. major*. Patton and Hindle (1928) considered *P. chinensis* to be a sub-species of *P. major* but the differences between the important characters in the two sandflies are very striking and constant.

FEMALE.

Size : Specimens from China and Syria 2.6 mm. to 3.2 mm. Specimens from Palestine 1.6 mm. to 1.8 mm.

Palps : 1, 4, (2, 3), 5. In one specimen 1, (2, 3, 4), 5.

Antennae : Segment 3 > 4 + 5. 3 < 4 + 5 + 6.

Pharynx (Plate IV, figs. 5-6) : The toothed area in the pharynx is triangular, the apex of the triangle being anterior. The teeth in optical section appear as fine wedges. They are more numerous and smaller than those of *P. sergenti*.

Spermathecae (fig. 3, e) : The spermathecae are much larger than those of *P. major*. They are spindle-shaped, feebly chitinated and the segmentation is incomplete and very faint. Anteriorly there is a short process bearing a tuft of hairs. The ducts are very wide and feebly chitinated.

Wings (fig. 5) : The wings show considerable variations. In specimens from China and Syria the wing index a/β varies from 1.75 mm. to 2.3 mm., in specimens from Rosh Pinah in Palestine from 1.3 mm. to 1.6 mm.

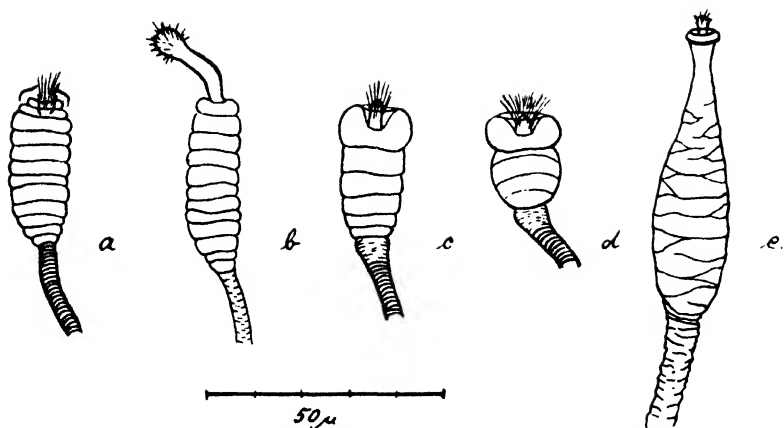


FIG. 3. Spermathecae of (a) *Pblebotomus papatasi*; (b) *Pblebotomus major*; (c) *Pblebotomus sergenti*; (d) *Pblebotomus sergenti* var. *mongolensis*; (e) *Pblebotomus chinensis*.

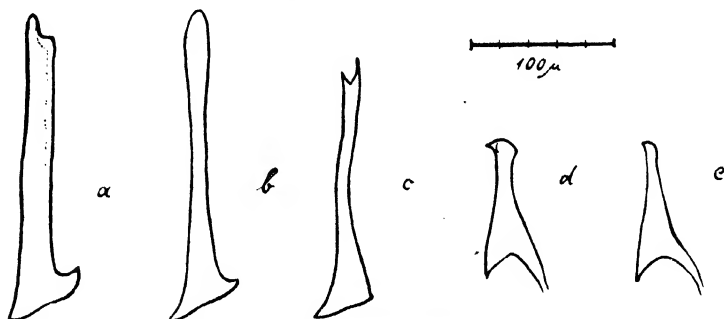


FIG. 4. Intromittant organ of (a) *Pblebotomus chinensis* from Syria. Dotted line indicates *P. chinensis* from China. (b) *Pblebotomus major*; (c) *Pblebotomus major* var. *perniciosus*; (d) *Pblebotomus sergenti* var. *mongolensis*; (e) *Pblebotomus sergenti*.

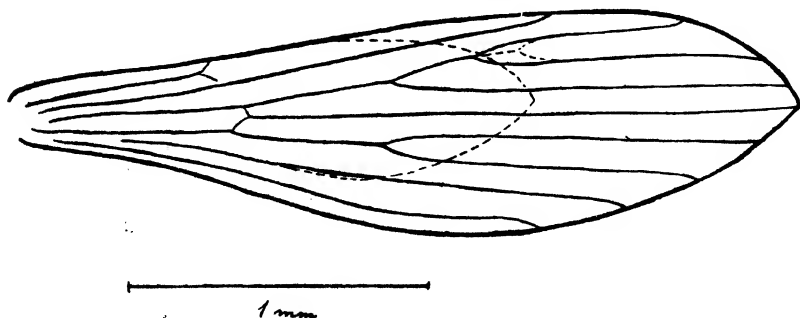


FIG. 5. Wing of *P. chinensis* from Aleppo, showing variation in wing index. The dotted outline of the small wing gives the size of specimens from Rosh Pinah as compared to those of Aleppo.

MALE.

Size, palps, antennae, pharynx and wings as in the female.

External genitalia : These are in general outline very similar to those of *P. major*. Sinton (1928) pointed out several differences between the genitalia of *P. chinensis* and *P. major*, namely the presence of a tubercle on the intromittant organ of *P. chinensis*. This tubercle is rather strongly developed in specimens from Aleppo, in specimens from China it is much flatter and situated further away from the tip (fig. 4, a).

It is probable that the specimens captured in Rosh Pinah represent a local race for they are much smaller than those from China and Syria. A comparison of specimens from China presented by Professor Patton and Dr. Young, two specimens from India presented by Major Sinton, and of thirteen specimens from Syria with those from Rosh Pinah showed that the former varied from 2.6 mm. to 3.2 mm. in size, and the latter from 1.6 mm. to 1.8 mm. There were no intermediate forms.

P. chinensis has previously been recorded from Northern China (Newstead, Patton and Hindle, Young and Hertig), from the Himalayas in India (Sinton, 1928) and from the Caucasus (Sinton, 1928). It therefore has a very wide distribution from Northern China to Syria. It is probably present in other parts of the Mediterranean basin but has been overlooked because of its superficial resemblance to *P. major*.

Distribution and material examined. Aleppo : 8 ♀♀, 4 ♂♂. Bcherreh : 1 ♀. Rosh Pinah : 8 ♀♀.

The four species described above fall into Sinton's erect-haired group. The table on page 279 shows their most important diagnostic characters.

Phlebotomus minutus Rondani

This species has been re-described by the authors in 1927. For comparison with other members of the *minutus* group described in this paper further data are given. In Mesopotamia some females were found which did not have a pigmented area in the buccal cavity. The palp formula was usually 1, 2, (3, 4), 5 or 1, 2, 4, 3, 5, and exceptionally, 1, 2, 3, 4, 5. The difference between 3 and 4 may be only 5μ.

In the antennae segment 3 was always smaller than 4 + 5.

TABLE I.

Table of diagnostic characters for the four species of the erect-haired group.

	<i>P. papatasi.</i>	<i>P. major.</i>	<i>P. chinensis.</i>	<i>P. sergenti.</i>
Pharynx ...	The teeth in optical section appear as horizontal scales or a network of lines. The pharynx is similar in both sexes.	The teeth in optical section appear as parallel rows of points. Pharynx similar in both sexes.	The toothed area is triangular, the teeth in optical section appear as fine wedges. Pharynx similar in both sexes.	FEMALE.—Posteriorly the teeth appear as horizontal scales. Anteriorly as strongly marked broad teeth with their axis longitudinal or slight oblique. MALE.—Narrower than that of the female, teeth smaller. Laterally teeth extend further forward than medially.
Spermathecae ...	Cylindrical and containing from eight to eleven segments. The superior segment is surmounted by a tuft of hairs. The ducts are narrow and heavily chitinated.	Cylindrical and contain about twelve segments. Anteriorly there is a long thin process with a club-shaped termination bearing a tuft of hairs. The ducts are narrow and lightly chitinated.	Spindle-shaped and much larger than those of <i>P. major</i> . Indications of incomplete segmentation. Anteriorly a short process standing on a broader ring bearing a tuft of hairs. The ducts are wide and lightly chitinated.	Consist of five or six segments. The superior segment is rounded. It is much longer and wider than the others and has a short process bearing a tuft of hairs.
Male Genitalia ...	Second segment of superior clasper longer than inferior clasper. Three terminal and two medial short spines. Intermediate appendage with two accessory appendages.	Second segment of superior clasper shorter than inferior clasper. Three medial and two terminal long spines. Intromittant organ with rounded end.	Second segment as in <i>P. major</i> . Intromittant organ bearing tubercle near the tip ventrally.	Second segment very short bearing two terminal and two medial long spines.

In the male genitalia the first segment of the superior clasper is constantly more than twice as long as the second. The termination of the intermediate appendage is blunt or club-shaped (fig. 6, f). The intromittant organ is blunt and broad and notched dorsally near its tip. This species is the most widely distributed and commonest of the *minutus* group.

Distribution and material examined.

PALESTINE:—Jerusalem: 1. Jericho: 150. Jaffa: 60. Rosh Pinah: 10. Tiberias: 8. Haifa: 20. (Males and females.)

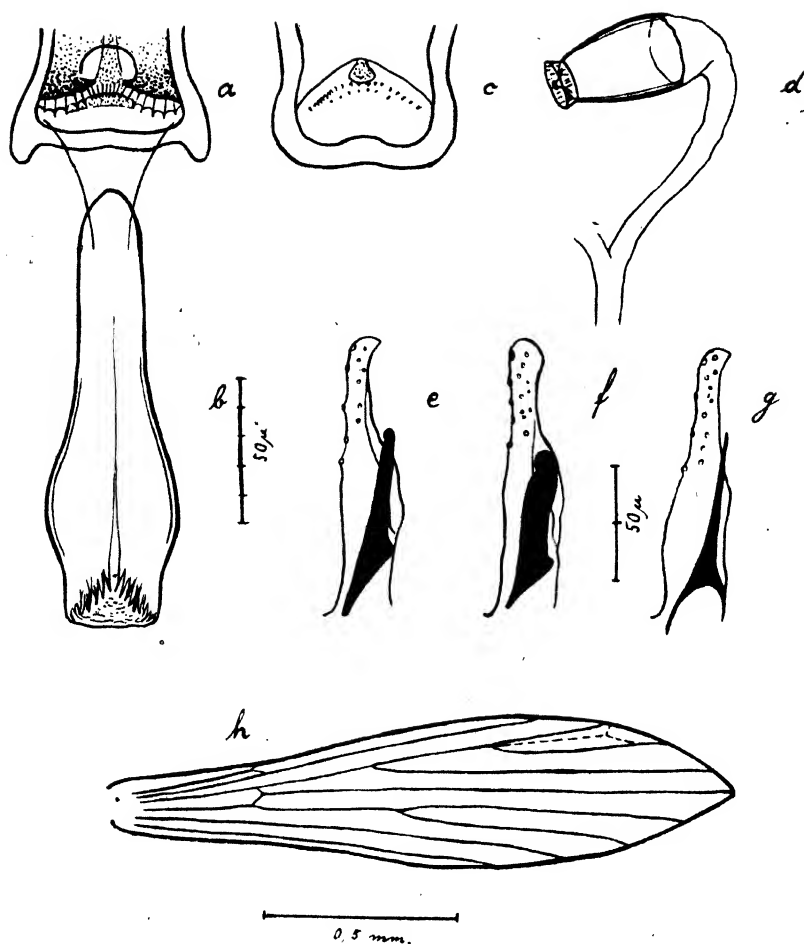


FIG. 6. *Phlebotomus baghdadis*, n.sp. (a) Buccal cavity of female; (b) Pharynx of female; (c) Buccal cavity of male; (d) Spermatheca.

Intermediate appendage with intromittant organ of (e) *Phlebotomus baghdadis*; (f) *Phlebotomus minus*; (g) *Phlebotomus africanus*; (h) Wing of *P. baghdadis* showing variation of wing index.

MESOPOTAMIA :—Baghdad : 70 ♀♀, 31 ♂♂. Mosul : 1 ♂.
Basrah : 17 ♂♂, 2 ♀♀.

SYRIA :—Aleppo : 2 ♀♀, 1 ♂. Bcherreh : 2 ♀♀. Addeh : 2 ♀♀.
Bar Elias : 1 ♂, 3 ♀♀. Zachleh : 1 ♀. Batrun : 2 ♀♀. Kubbah :
2 ♂♂. Enfeh : 1 ♂, 5 ♀♀.

In Baghdad specimens were frequently found feeding inside the ears of geckoes where they remained as long as six hours.

Phlebotomus minutus var. *niger*

We found this variety only in Ben Shemen, Palestine, on a poultry farm.

Phlebotomus africanus Newstead

This species has been sufficiently described. It is common in Palestine but was not found in Syria and Mesopotamia. This sandfly is in our opinion essentially an East African species and Palestine represents its northern limit. We found it in material from Stanleyville, Congo, sent by Dr. J. Schwetz. It is probably absent from North-West Africa. Among a large collection of sandflies from Algeria sent by Dr. L. Parrot we found only two species, *P. minutus* and *P. parroti*. The latter species has probably been confused with *P. africanus* owing to the similarity of the palp formula in the two species.

Material examined. Jericho : 100. Jaffa : 40. Haifa : 10.
(Males and females.)

Phlebotomus palestinensis Adler and Theodor (1927)

This species is very rare. We found one female in Jericho and three females in Baghdad.

Phlebotomus baghdadis n.sp.

This sandfly is about as common in Baghdad as *P. minutus*. It is unequally distributed and was found to be very common in some parts of Baghdad and absent in others. The female feeds on geckoes and birds.

FEMALE.

Size : 1.7 mm. to 2 mm.

Palp formula : 1, 2, (3, 4), 5.

Antennae : Segment 3 = 4 + 5. This distinguishes it from

P. minutus, in which segment 3 is always smaller than $4 + 5$.
 $3 < 12 \pm 16$.

Segment 4 is in the majority of the specimens exactly half as long as 3.

Wings (fig. 6, *h*): The wing characters are variable. α is generally shorter than β , but in some specimens α was equal to β , the wing index being 1. In the majority of the specimens the wing index α/β was between 0.5 mm. and 0.75 mm. The termination of the first vein also varied. In some specimens it covered the half length of α , in others it was negative. $\alpha = 0.13$ mm. to 0.3 mm. $\beta = 0.3$ mm. $\gamma = 0.22$ mm. to 0.33 mm. $\delta = -0.22$ mm. to $+0.14$ mm.

Buccal cavity (fig. 6, *a*): Pigmented area rather faint, roughly triangular, sometimes absent. The armature consists of a row of sixteen to eighteen rather broad pointed teeth standing on an arc concave posteriorly. The teeth in the middle are narrower than the side ones. The appearance of the teeth depends to some extent on the way the specimen is mounted. In preparations where the buccal cavity has been dissected out, the long points of the teeth are visible, in heads mounted complete they are sometimes not evident. The plate joining the two lateral bars of the buccal cavity shows a deep notch which is about three-quarters of a circle with a narrow opening. The proximal border of the plate has several blunt processes. This plate is rather heavily pigmented for some distance beyond the notch. Sinton figures a similar but much smaller notch in *P. babu* and a very shallow notch is present in *P. shorttii*.

Pharynx (fig. 6, *b*): The pharynx is rather narrow; posteriorly it is about twice as wide as anteriorly. The two side plates bear very fine slender teeth, the dorsal plate minute short teeth.

Spermathecae (fig. 6, *d*): They are similar in outline to those of *P. africanus* but they are heavily chitinised only in the superior three-quarters of the capsule and the duct is very wide and feebly chitinised.

MALE.

Palps and antennae as in the female.

Wings: The variation mentioned in the female is still more pronounced in the male, the wing index α/β varying from 1 mm. to 0.26 mm. In one specimen, which is probably abnormal, the wing

index was 0.03 mm., i.e., a was thirty times as long as β (fig. 6, h) ($\beta = 0.0176$ mm. ; $a = 0.53$ mm.).

Buccal cavity (fig. 6, c): In the female the broad plate which bears the notch is deeply angulated and a trace of a pigmented area was visible in some specimens. There are a few very fine pointed teeth standing on two or three curved lines but they are only visible if the buccal cavity is dissected out and even then appear to be absent in some specimens.

Pharynx: The pharynx is similar in general shape to that of the female. It has no teeth but only very faint ridges. This distinguishes it from the pharynx of the male of *P. minutus* which has many short teeth.

External genitalia: They are very similar to those of *P. minutus* but show some constant differences. The first segment of the superior clasper is nearly always exactly twice as long as the second, while in *P. minutus* it is always more than twice as long. The end of the intermediate appendages is sharply pointed ventrally while in *P. minutus* the termination of this appendage is blunt or sometimes club-shaped. The intromittant organ tapers uniformly and has a blunt end (fig. 6, e). In *P. africanus* the intromittant organ ends in a sharp point (fig. 6, g).

Diagnosis. This species is readily distinguished from *P. minutus* by the pharynx, buccal cavity and spermathecae in the female, as well as by the third antennal segment, which is relatively longer than in *P. minutus*. The male can be distinguished by the very small teeth in the buccal cavity, antennal formula and some slight differences in the genitalia. Systematically this species is closely related to *P. shorttii* and *P. babu*. From *P. shorttii* it differs in the shape of the notch and the teeth in the buccal cavity in both sexes. From *P. babu* it differs in the number of teeth in the buccal cavity (about thirty in *P. babu*) and the antennal formula.

Distribution and material examined. Baghdad: 130 ♀♀, 22 ♂♂. Basrah: 1 ♀, 3 ♂♂.

Types in the Microbiological Institute, Hebrew University, Jerusalem.

Phlebotomus iraqi, n.sp.

Size : 2 mm.

Palp formula : 1, 2, 3, 4, 5.

Antennae : Segment 3 < 4 + 5.

Wings : Index $a/\beta = 1$. δ = First vein covering half the length of a . $a = 0.27$ mm., $\beta = 0.27$ mm., $\gamma = 0.32$ mm., $\delta = 0.15$ mm.

Buccal cavity (fig. 7, a) : Pigmented area absent. The teeth (about fifty) stand on an arc convex posteriorly ; they are parallel and have short blunt points at both ends. In front of and parallel to the main ridge of teeth there is a second row of very small blunt teeth.

Pharynx (fig. 7, b) : Its widest part posteriorly is four times as wide as the narrow anterior part. It bulges out very suddenly and narrows again. Posteriorly there are many small slender teeth pointing backwards.

Spermathecae (fig. 7, c) : Wide tubes without any crenulation. Similar to those of *P. minutus*.

Male unknown.

Diagnosis. This species is very closely related to *P. squamipleuris* which it resembles in every external character and in the structure of the buccal cavity. The pharynx of *P. squamipleuris* does not show the strong bulge nor the sudden narrowing in the posterior part. The spermathecae of *P. iraqi* are very different from the spine bearing spherical spermathecae of *P. squamipleuris*. *P. iraqi* is also closely related to *P. squamirostris* Newstead (Syn. *P. taianensis* Patton and Hindle). There are some slight differences in the buccal cavity. The spermathecae of *P. squamirostris* are much narrower than those of *P. iraqi* ; they have narrow ducts and show indications of segmentation superiorly.

Material. Baghdad : 1 ♀.

Type in the Microbiological Institute, Hebrew University, Jerusalem.

Phlebotomus sp. near *clydei* Sinton.

A rather small, light coloured species.

Size : 1.5 mm. to 1.8 mm.

FEMALE.

Palp formula : 1, 2, 4, 3, 5.

Antennae: Segment 3 < 4 + 5.

Wings: Index $a/\beta = 0.5$ mm. to 0.7 mm. δ usually 0 mm., sometimes slightly positive. $a = 0.11$ mm. to 0.15 mm., $\beta = 0.22$ mm. to 0.26 mm., $\gamma = 0.22$ mm. to 0.28 mm., $\delta = 0$ mm. to 0.046 mm.

Buccal cavity (fig. 8, a): Pigmented area heart-shaped, very faint. Sixteen to seventeen teeth standing on a line very slightly convex anteriorly. The teeth are pointed and directed towards the middle of the buccal cavity. Behind them there is a row of small indentations.

Pharynx (fig. 8, b): The pharynx appears to be entirely unarmed. Posteriorly it is less than twice as wide as anteriorly.

Spermathecae (fig. 8, e): They are segmented and very similar to those of *P. papatasii* but smaller. They have about six to eight segments and narrow ducts which terminate separately.

MALE.

Palp, Antennae, Wings as in the female.

Buccal cavity (fig. 8, c): A row of twelve to thirteen pointed teeth, similar in general arrangement to those of the female.

Pharynx: Narrower than in the female, otherwise similar.

External genitalia: Minutus type. The first segment of the superior clasper is twice as long as the second. The intermediate appendage ends in a sharply-pointed beak-like process. The intromittant organ ends in a very sharp point (fig. 8, d).

Diagnosis. This species resembles *P. clydei* very closely. The number and shape of the teeth in the buccal cavity of the male seem to be different. Some slight differences may exist in the pharynx which, posteriorly, is only 1.75 times as broad as anteriorly and not three times as in *P. clydei*. We found no armature in the pharynx whereas Sinton figures toothed ridges in *P. clydei*. In the male genitalia the first segment of the superior clasper is exactly twice as long as the second, while in *P. clydei* it is 2.3 to 2.4 times as long as the second segment. *P. clydei* is a rather big species (2.2 mm. to 2.9 mm.) while the Palestinian species is only 1.5 mm. to 1.8 mm. long. The wing of *P. clydei* is about three-fifths of the body length while in our specimens it was nearly as long as the body.

Unfortunately we had no specimens of *P. clydei* for comparison and cannot therefore determine the definite relationship of the Palestinian specimens to this species.

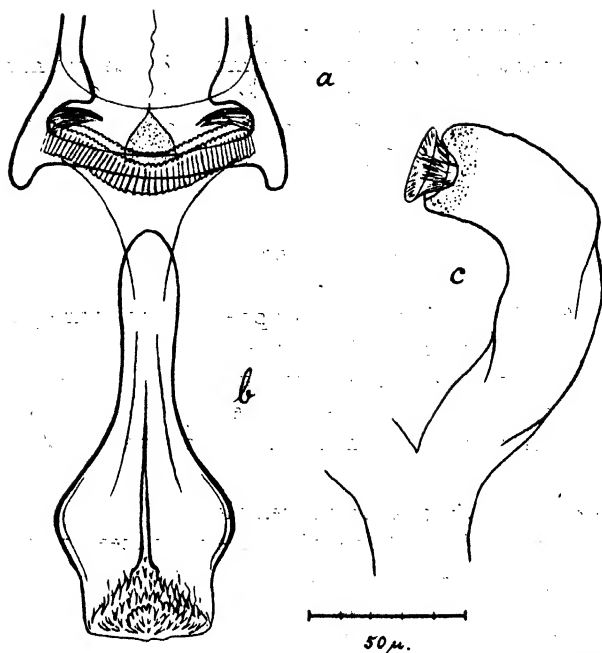


FIG. 7. *Plebotomus iragi*, n.sp. (a) Buccal cavity; (b) Pharynx; (c) Spermatheca.

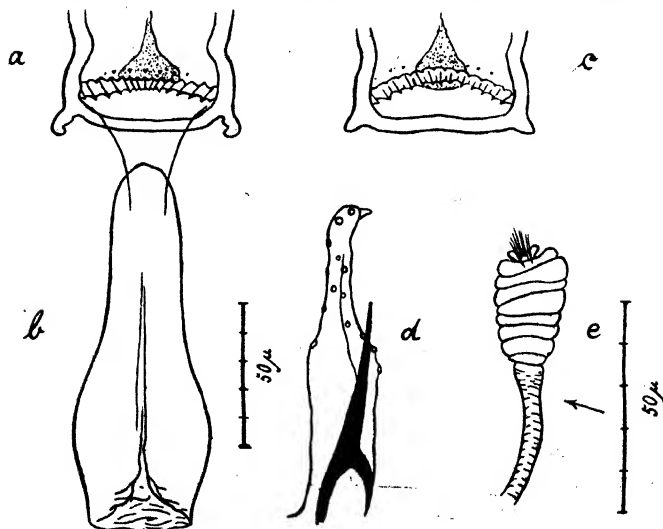


FIG. 8. *Plebotomus* sp. near *clydei* Sinton. (a) Buccal cavity of female; (b) Pharynx of female; (c) Buccal cavity of male; (d) Intermediate appendage with intromittant organ; (e) Spermatheca.

Material examined. 4 ♂♂, 2 ♀♀ from Tiberias, Palestine.

Types in the Microbiological Institute, Hebrew University, Jerusalem.

KEY TO THE SPECIES OF THE MINUTUS GROUP DESCRIBED.

(Females only).

1. Spermathecae segmented *P. near clydei*
 Spermathecae not segmented 2
2. Notch in posterior part of the buccal cavity *P. baghdadis*
 No notch in posterior part of the buccal cavity 3
3. Pharynx broad and heavily toothed posteriorly 4
 Pharynx slightly toothed posteriorly 5
4. Spermathecae tubular opening into a wide common duct.
 Teeth in buccal cavity stand on an arc markedly convex
 anteriorly *P. minutus*
 Spermathecae capsules opening into separate ducts. Teeth
 in buccal cavity stand on a straight line *P. palestinesis*
5. Spermathecae capsular. 40 to 50 narrow teeth in buccal
 cavity standing on an arc slightly convex anteriorly.
 Pharynx twice as wide posteriorly as anteriorly *P. africanus*
 Spermathecae tubular. Teeth in buccal cavity stand on an
 arc convex posteriorly. Pharynx posteriorly four times as
 wide as anteriorly *P. iraqi*

PARASITES OF SANDFLIES OTHER THAN LEISHMANIA

The following parasites were found :—

1. *Fungi*. A fungus parasitic in the coelome and in the ova was found in *P. papatasi* in Palestine, and in *P. papatasi* and *P. sergenti* in Mesopotamia and Syria. Infected eggs are completely destroyed. This fungus has been figured by us in 1927.

2. *Nematodes*. Nematodes were found in the haemocoel of *P. papatasi* and *P. sergenti*. Both eggs with morulae and active larvae were seen and free larvae were numerous in the haemocoel. In Baghdad two out of 683 *P. sergenti* and three out of 528 *P. papatasi* were found infected. The nematode larvae pass from the haemocoel into the ovary and may pass out together with the eggs. The larvae probably invade the sandfly larvae and undergo a cycle of development. Several laboratory bred sandflies were found infected.

3. *A Crithidia of P. baghdadis*. In five out of 78 ♀ specimens a *Crithidia* was found in the midgut. In four specimens the cardia and stomach were infected and in one specimen which showed a slight infection only the upper part of the cardia was invaded. In the

cardia the flagellates were found attached to the rhabdorium by their flagella.

Types of Parasites Found.

1. Rounded or irregular bodies without a flagellum (about 9μ in diameter) with a single nucleus and one or two blepharoplasts; they may have many protoplasmic vacuoles and chromatoid bodies (fig. 9, a-c).

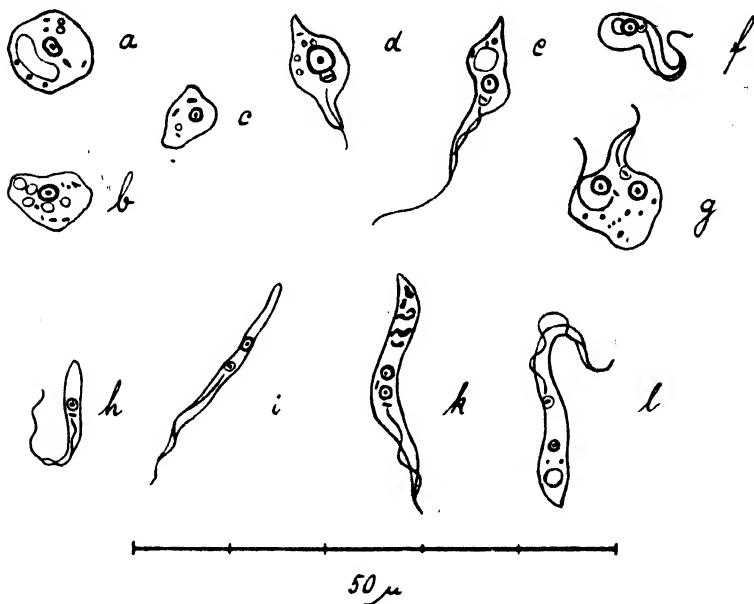


FIG. 9. A Crithidia of *P. baghdadis*. (a-c) Rounded forms without flagellum; (d-h) Short and intermediate flagellates with varying length of flagellum; (i-l) Long forms.

2. Short and intermediate flagellates with or without a free flagellum (10μ to 16μ). Some of these forms were vacuolated and contained chromatoid bodies (fig. 9, d-h).

3. Long flagellates (up to 27μ) (fig. 9, i-l). The free part of the flagellum may or may not be very short. These forms were found in the cardia. The blepharoplast is usually very close to the nucleus which is central or slightly posterior. A slightly eosinophile flagellar vacuole was present in all forms. The protoplasmic vacuoles when present were often very large, sometimes larger than the nucleus.

The chromatoid bodies were in some specimens very numerous and they were often much larger than the blepharoplast.

The fact that one specimen of *P. baghdadis* showed flagellates in the cardia and not in the stomach and hindgut indicates that transmission to the vertebrate host is by the bite of the insect, although infection by ingestion or infected sandflies is also to be expected. The vertebrate host is probably a gecko, though culture of the heart's blood of forty-seven geckoes in Baghdad gave negative results. Geckoes were occasionally observed eating sandflies.

Flagellates were inoculated into two scarified points on the left forearm of a human being (9.6.28). As was to be expected the result was negative.

4. *Mites*. Mites were occasionally found attached to the abdomen of *P. papatasi* and *P. sergenti* without apparently doing any harm to the sandflies.

5. *Sporozoa*. An oöcyst of a sporozoon, probably a *Hepatozoon* sp., was found in a single specimen of *P. papatasi* in Jericho, in 1925. This parasite has been previously described (1925). The result of the inoculation of sporozoites into two human beings is still negative.

THE RELATION OF SANDFLIES OF THE ERECT-HAIRED GROUP IN RELATION TO VISCERAL LEISHMANIASIS

In the case of Mediterranean visceral Leishmaniasis there is unfortunately very little experimental evidence to incriminate any particular species of *Phlebotomus*. The situation is further complicated by the fact that the distribution of species in the Mediterranean basin is not known with any degree of accuracy, for in all the publications on the sandflies of this region the diagnosis of species was based on insufficient data. In the area under discussion, visceral Leishmaniasis is confined to the Lebanon (see Map No. 1) where the following species occur.

P. papatasi. This species occurs everywhere in this region below the level of 1,650 metres.

P. major. This species is found in localities showing great topographical and climatical variations. It occurs in the plain at the foot of the Lebanon and in the hills up to a height of 2,000 metres. We found one female in a house in the ancient cedar grove near

Bcherreh, 2,000 metres above sea level. This grove is said to be under snow for six months in the year. *P. major* is relatively more common in the mountains than in the coastal plain.

P. chinensis. This species was found in Bcherreh (1,650 metres). It can exist within wide climatic ranges for it was also found in Aleppo which has a much hotter and drier climate than the Lebanon.

P. sergenti. This species was found in Aleppo and Bcherreh.

Of the above four species the only one which has been incriminated experimentally by feeding experiments on infected human beings and animals as a vector of visceral Leishmaniasis is *P. chinensis* (Patton and Hindle, Young and Hertig).

We have found this species in Aleppo, Bcherreh, the Lebanon and in Rosh Pinah near Lake Tiberias, where visceral Leishmaniasis is unknown, or is so rare that it has been overlooked.

The other three species cannot be excluded definitely as possible carriers although their distribution does not coincide with that of the disease. Sinton (1925) suggested *P. major* var. *perniciosus* as a possible carrier of visceral Leishmaniasis in the Mediterranean because it is closely related to *P. argentipes* but we did not find this variety in the area examined. *P. major* occurs in places where the disease is unknown, e.g., Jerusalem, Mozah near Jerusalem, Rosh Pinah, Haifa.

P. papatasi. This species occurs in all Mediterranean foci of visceral Leishmaniasis, but it also occurs in enormous numbers in places where the disease is unknown, e.g., Jerusalem, Jericho, Haifa, Jaffa, Rehoboth (all in Palestine), Baghdad, Basrah, Mosul.

The behaviour of *L. infantum* in the sandfly suggests that *P. papatasi* may be a carrier, for the authors have shown (1927) that cultures of a strain of *L. infantum*, when ingested by *P. papatasi*, multiplied and adopted an anterior position. Since then several more strains have been found to behave similarly. Further a strain of canine visceral Leishmania presented by Professor Nicolle of the Pasteur Institute, Tunis, produced local lesions on inoculation into the tail of mice. Laboratory bred *P. papatasi* fed on these lesions and some became infected, the flagellates tending to adopt an anterior position. Both in the experiments with cultures and in the direct feeding experiments on a mouse an anterior position was only adopted when the resulting infection in the sandfly was

very heavy. In this respect *L. infantum* differs from *L. tropica* which tends to adopt an anterior position in *P. papatasi* and *P. sergenti* even when the resulting infection is very slight. It was also shown (1927) that the infection rate of *P. papatasi* with *L. infantum* from cultures was diminished if the cultures were emulsified in specific immune serum.

This is up to the present the only experimental evidence relating Mediterranean visceral Leishmaniasis with sandflies. It tends to show that *P. papatasi* could be infected in nature only under very favourable conditions, i.e., by feeding on a host with a large number of parasites in the circulating blood. In the absence of direct feeding experiments on human beings we concluded that *P. papatasi* may transmit the disease but rarely.

P. sergenti. This species occurs in some foci of Mediterranean visceral Leishmaniasis and is also present in parts free from the disease, e.g., Baghdad, where it is very common. There is no experimental evidence either for or against this species being a carrier. Patton and Hindle (1928), working in China, showed that *L. donovani* from experimentally infected hamsters exflagellates in *P. sergenti* var. *mongolensis* but that the flagellates usually remain in the stomach, very rarely ascend the cardia and never proceed anteriorly beyond the cardia. This variety is therefore either a very poor carrier or does not carry at all. It is uncertain whether these observations can be applied to *P. sergenti*.

Sinton (1925) was the first to correlate the distribution of *Phlebotomus* sp. and *Leishmania* sp. This method in Sinton's hands at once gave valuable results for it implicated *P. argentipes* as the carrier of Kala-Azar and *P. sergenti* as the carrier of oriental sore, but it has its limitations in the case of *L. tropica* and possibly also in a case of visceral Leishmaniasis. Strains of *L. tropica* vary enormously in their infectivity for *P. papatasi* and the visceral Leishmanias probably also vary in their infectivity for various sandflies, e.g., cultures of several strains of Mediterranean visceral Leishmanias were found to be more infective for *P. papatasi* than cultures of two strains of *L. donovani* from India. Further, *L. donovani* bodies from cultures on immune serum exflagellated frequently in *P. papatasi* in the case of the Mediterranean strains, rarely in the case of the Indian strains (we will deal with this point more fully

in another communication). The differences in infectivity of various strains of *Leishmania* for *Phlebotomus* sp. probably account for the discrepancies between the distribution of the insect vector and the protozoon.

Hindle (1928) showed that Chinese strains of visceral Leishmaniasis also vary in their infectivity for sandflies and hamsters; the infectivity for sandflies and for hamsters are two individual factors not necessarily correlated, for a strain may have a low infectivity for hamsters and at the same time a very high infectivity for sandflies and vice versa. Hindle's observations are not strictly comparable to ours for whereas we estimated infectivity by feeding sandflies on washed cultures Hindle fed his sandflies on infected hamsters. Hindle's method involves an unknown factor, namely the influence of body juices of a vertebrate on the ingested *Leishmania* for it has been shown that in *P. papatasi* the infection rate of a strain of *L. infantum* was reduced in the presence of specific agglutinins. Nevertheless the number of strains and the large number of animals employed by Hindle in his experiments are sufficient to prove variation in infectivity of strains both for hamsters and sandflies. There may be other factors involved such as differences between races of *Phlebotomus* but whereas these are hypothetical the variations in strains of *Leishmania* are a fact established experimentally beyond doubt. It follows that in addition to the valuable evidence adduced from the distribution of the disease and various species of sandflies every focus should be examined experimentally with regard to the behaviour of the local strains in the local sandflies.

The distribution of visceral Leishmaniasis in the Mediterranean basin suggests that the disease is either transmitted by a species of insect which is common and widely distributed but is not an efficient vector, or that it is transmitted by an efficient vector which is comparatively rare and irregularly distributed, possibly *P. chinensis*. The discovery of the latter species in a part of the Mediterranean where visceral Leishmaniasis occurs is therefore of great significance.

No further conclusions can be drawn until more sandfly surveys of endemic foci based on modern methods of diagnosis are carried out and correlated with feeding experiments on man and animals.

THE DISTRIBUTION OF SANDFLIES OF THE ERECT-HAIRED GROUP IN RELATION TO ORIENTAL SORE

Foci of cutaneous Leishmaniasis are of two types.

(A) Big endemic centres such as Baghdad, where few inhabitants escape the disease.

(B) Areas where there are a number of sporadic cases annually but where the bulk of the population escapes the disease.

FOCI WITH SPORADIC CASES

Sporadic cases of oriental sore occur in the following places in Palestine and Transjordan.

Jerusalem and its environments. A few endemic cases occur annually although there are always a number of imported cases from Persia, Baghdad and Aleppo. Dostrowsky (1926), who studied oriental sore in Palestine, came to the conclusion that the endemic cases in Jerusalem are not acquired through direct contact with the imported ones.

Artuf. Oriental sore first appeared in this village in 1923.

Kantara. Kligler (1923) reported three cases from Kantara.

Jericho. This is the largest endemic focus. Oriental sore has been known for a very long time in this town.

Amman. According to Dr. Blofield cases of oriental sore are rather common in Amman, Djerash and the neighbouring villages. We saw several locally acquired sores in people who never left their village.

In Jericho *P. papatasii* is very common and is the only sandfly which feeds on man. In the other above-mentioned places we found *P. papatasii* and *P. major*. There is abundant evidence to incriminate *P. papatasii* as a vector of oriental sore but *P. major* has not yet been investigated experimentally. It is not present in Jericho or in Baghdad but as previously pointed out this negative evidence is insufficient.

In Syria sporadic cases occur in the coastal plain of the Lebanon where *P. papatasii* and *P. major* are common. We found *P. sergenti* in Bcherreh (one female) but oriental sore is unknown in this village although we saw one imported case from Aleppo.

LARGE ENDEMIC CENTRES

BAGHDAD. This famous focus of oriental sore was investigated in detail. We commenced work in Baghdad on May 13th, 1928, and continued with two interruptions up to July 12th. The method of work was as follows: Baghdad was divided into districts and a number of houses were examined in each one. As many sandflies as possible were collected in each district and taken to the Central Laboratory where they were dissected and diagnosed. At the same time observations were made on the prevalence of oriental sore in each district and these observations were co-related with the numbers and species of sandflies present. In all quarters examined the proportion of *P. sergenti* to *P. papatasi* was determined by dissection of caught females. The area examined included Baghdad, Muadham, a small town five kilometres north of Baghdad, the Royal Air Force Camp at Hunaidi, south of Baghdad, and the agricultural experimental station at Rustamiyah.

The only two species which come into consideration as possible carriers in Baghdad are *P. sergenti* and *P. papatasi*. *P. major* and *P. chinensis* were not found.

Owing to the structure of the houses it is difficult to form a relative quantitative estimate of sandflies in Baghdad. The houses are built of bricks, the walls in many houses are not plastered and the interstices in the walls form an admirable refuge for sandflies when disturbed. On several occasions we saw large numbers of sandflies disappear quickly into cracks in the walls after being disturbed by moving a few chairs. An examination conducted a few minutes later showed very few sandflies in the room. The insects are not common in the upper stories and in rooms occupied during the day. Most houses have a large cellar (sardab) originally intended for shelter during the heat of the day but often used as a lumber room. During the day sandflies seek shelter and humidity in dark cool rooms and in the cellar inside holes in the walls where they are extremely difficult to find and capture. During summer the inhabitants sleep on the roof and the sandflies after feeding enter cracks in the walls of the courtyard where they are almost impossible to detect. Sandflies are common in bathrooms (hammams) which are dark, moist and badly ventilated, and unless the cellars and bathrooms are

examined, few sandflies are seen. In modern houses with smooth walls and without cellars sandflies are found in bedrooms and living-rooms where they are easy to detect and capture.

The survey showed that the distribution of *P. sergenti* and *P. papatasi* in Baghdad is very unequal.

In Haidar Khan and in the Jewish and Armenian quarters where scarcely anyone escapes infection, *P. sergenti* is the common species and *P. papatasi* is so rare that it can be safely excluded as the local carrier.

In the Senhac district where oriental sore is also very common *P. papatasi* and *P. sergenti* are equally numerous (in some parts *P. sergenti* predominates).

Three Armenian compounds in Senhac were specially examined. In one where about fifty families lived and every child had either oriental sore in active form or recently healed scars of the disease, the two species were equally common. In another compound inhabited by six families where every individual was infected, sandflies were very few. Only twelve females, *P. papatasi*, were caught and about twenty males of the same species were seen on the walls. (With a little practice it is easy to distinguish males of the two species at sight owing to the fact that the male of *P. sergenti* has relatively short external genitalia and the posterior part of the body is less curved than that of *P. papatasi*.) In a third compound inhabited by over one hundred families oriental sore was common, many children were infected but a large number showed no signs of the disease. In this compound *P. papatasi* was common and *P. sergenti* rare.

South-east of Senhac there is a large Armenian encampment with a population of about two thousand. There were several cases of oriental sore among children but the disease was found to be rare. *P. papatasi* was the prevalent species in this quarter and *P. sergenti* was not found.

Examining this comparatively small area including Senhac and the Armenian encampment we were struck by the curious fact of the proximity of a district where the bulk of the population was infected to another where the disease is rare. The Armenian Camp is about 600 metres from Senhac; between the two there is an open area containing no habitations. This observation is in accord with the

theory of transmission by a biting insect with a very limited range of movement.

Bed bugs are rare in Baghdad and the only biting insect which is common and restricted in its range is the sandfly.

In Alwayah a new district occupied by government officials sandflies are very numerous, probably more so than in the rest of Baghdad. In this district *P. papatasii* is six times as numerous as *P. sergenti*. Cases of oriental sore have not yet appeared in Alwayah.

The Royal Air Force Camp at Hunaidi, south of Baghdad, is heavily infested with *P. papatasii*. *P. sergenti* is very rare. Cutaneous Leishmaniasis is absent but sandfly fever is very common.

The area round the Royal Hospital is heavily infested with *P. papatasii*; *P. sergenti* is scarce. Cases of oriental sore are not uncommon but they are not nearly as numerous as in Haidar Khan or Senhac and a large number of children escape the disease.

The town of Muadham, five kilometres north of Baghdad, was carefully examined. The whole population has either active oriental sore or old scars of the disease. Both *P. sergenti* and *P. papatasii* were found, the former being the commoner species.

In the thickly populated district of Baghdad West where practically no one escapes the disease, *P. sergenti* and *P. papatasii* are both common, the former species predominating. Proceeding southwards along the bank of the river the proportion of *P. papatasii* to *P. sergenti* increases until in the southernmost suburb, i.e., the Railway Quarters, *P. papatasii* is very common and *P. sergenti* is rare. In this district many cases of cutaneous Leishmaniasis have occurred but many people escape the disease.

The distribution of the two species in Baghdad shows that in the greater part of the city *P. sergenti* is the main carrier and that in districts where this species predominates practically the whole population is infected; where *P. papatasii* predominates cases occur but many people escape infection. We conclude therefore on evidence of distribution that *P. sergenti* is a more suitable carrier of the strains of *L. tropica* prevalent in Baghdad than *P. papatasii*. This difference between the two species may depend partly on the fact that *P. sergenti* feeds more readily on injured than on normal skin and partly on the strains of *L. tropica* prevalent in Baghdad.

That it is not due to any racial peculiarities of the local *P. papatasii* is proved by the fact that wild specimens of *P. papatasii* caught in Baghdad were easily infected by feeding through membranes on a strain of *L. tropica* from Palestine which had been previously examined in Jerusalem and found to be highly infective for *P. papatasii*; again a strain of *L. tropica* isolated from a naturally occurring case in Baghdad showed a low infectivity for *P. papatasii* bred from wild females caught in Jerusalem and Jericho.

The peculiar local distribution of the two species in Baghdad appears to depend on the surroundings of the houses. Wherever houses are surrounded by gardens or plantations with a soft soil the predominating species is *P. papatasii*. In districts such as Haidar Khan where the houses are close together and there are no gardens *P. sergenti* predominates. This suggests that *P. papatasii* prefers to lay its eggs in soft soil in the neighbourhood of houses, while *P. sergenti* prefers to lay eggs inside houses, probably in the cellars.

Mosul is another great endemic centre of oriental sore. Both *P. papatasii* and *P. sergenti* occur, the former species being commoner in the houses along the river bank. The time at our disposal did not permit a survey of sandflies and oriental sore in every district of the town (of a total of sixty-two sandflies—♀♂—caught, forty were *P. papatasii* and twenty-two were *P. sergenti*).

It is interesting to compare conditions in Mosul and Baghdad with those of Basrah and district where oriental sore is either absent or rare. Sandflies which feed on man are apparently rare in Basrah. We found only two females, *P. papatasii*, during four days' collecting in Basrah town and twenty-seven females in Ashar and Makinah. *P. sergenti* was not found. The minutus group is not uncommon. In Ashar sandflies are rare throughout the greater part of the town but they are not uncommon in a limited area about the centre of the town. *P. papatasii*, *P. minutus* and *P. baghdadis* were found but not *P. sergenti*. In Makinah near Basrah where *P. papatasii* was found to be rather common there is little or no endemic oriental sore. Zobeir, a small town in the desert about 15 kilometres from Basrah, was examined. The ground is hard and dry, the relative humidity of the air is very low and the temperature is even higher than in Basrah. There is no local water supply and water is brought into the town on mules from a spring several miles distant.

No sandflies or mosquitos were found in the town which is free from oriental sore.

ALEPPO. This endemic centre was examined between August 10th and September 12th, 1928. The examination was conducted on similar lines to those employed in Baghdad. The following species of sandflies were found :

P. papatasii : 570 ♀♀ (males of this species which are easily diagnosed at sight were not collected).

P. sergenti : 111 ♀, 50 ♂♂.

P. major : 1 ♀.

P. chinensis : 8 ♀♀, 4 ♂♂.

It will be seen that *P. papatasii* and *P. sergenti* are the common species in Aleppo, the former being by far the commoner. In one district only (Bachsita) *P. papatasii* and *P. sergenti* were found in almost equal numbers, but the population of this district was not more heavily infected with oriental sore than that of other districts on the outskirts of the town, where *P. papatasii* is about ten times as common as *P. sergenti*.

P. major and *P. chinensis* are too rare to be considered as carriers in Aleppo where the disease is very common.

Unlike Baghdad the distribution of the disease in Aleppo does not correspond particularly to the distribution of *P. sergenti*. In the absence of experimental data and on the evidence of the distribution of the two important species we conclude that both *P. papatasii* and *P. sergenti* carry the disease in Aleppo.

BAR ELIAS. This village lies in the plain between Lebanon and Antilebanon, about 25 kilometres south-west of Baalbek. Until six years ago oriental sore was unknown in the village. According to the statement of the local sheikh an epidemic followed the return of an infected villager who had resided for some time in Aleppo where she acquired 'Aleppo button.' During the next five years almost all the population, adults and children, acquired the disease. During the last twelve months no more cases occurred probably because every susceptible person had already been infected within the previous five years. In Bar Elias sandflies are very numerous ; only three species were found : *P. papatasii*, *P. major* and *P. minutus*. *P. sergenti* was not found. *P. major* and *P. minutus* were very rare but *P. papatasii* was present in enormous numbers ; *P. papatasii*

is therefore the only possible carrier to be considered. Bar Elias is a striking example of a heavily infested focus of oriental sore where *P. sergenti* is absent. It is interesting to note that the neighbouring villages are entirely free from oriental sore.

The curious distribution of oriental sore in relation to the two carriers can only be explained on the ground of variations in infectivity of different strains of *L. tropica* for the insect vectors. The epidemiological evidence collected in Baghdad proves clearly that here we are dealing with strains which are far more infective for *P. sergenti* than for *P. papatasi*. In Bar Elias we are dealing with strains highly infective for *P. papatasi*. In Aleppo there is not complete evidence for comparing the infectivity of the local strains in the two species, whereas in Palestine, where *P. sergenti* does not occur, the distribution of the disease shows that the dominant strains are not highly infective for *P. papatasi*. There are as yet few data for comparing the infectivity for the two sandflies of strains from places where there are sporadic cases of oriental sore. In one case from Artuf, where *P. papatasi* is abundant and *P. sergenti* absent, feeding experiments showed a higher infection rate in *P. sergenti* than in *P. papatasi* (58 per cent. in *P. sergenti* and 16 per cent. in *P. papatasi*). On the whole the evidence shows that *P. sergenti* is a better carrier than *P. papatasi* but that some strains are transmitted very readily by *P. papatasi*.

Attention must be drawn to the absence of oriental sore in localities where *P. sergenti* and *P. papatasi* occur, e.g., Alwayah where both species are common, Haifa where *P. papatasi* is abundant, Bcherreh where both species occur.

THE INFECTION RATE OF WILD SANDFLIES WITH LEISHMANIA

The determination of the infection rate with *Leishmania* in wild sandflies is of value only if the distribution of the flagellates in the sandfly is observed. The *Leishmanias* of man all tend to adopt an anterior position in the sandfly. *L. tarentolae* also tends to adopt an anterior position in *P. papatasi* whereas *L. ceramodactyli* adopts a posterior position, but if the infection is very heavy the infection may also extend anteriorly. A further complication exists owing to the fact that in early infections the flagellates are confined to the

stomach and it is therefore impossible to distinguish between *Leishmanias* with an anterior and those with a posterior position. The sandflies which feed on man also feed on animals; thus we caught *P. papatasi* and *P. sergenti* in large numbers in fowl sheds, and on dissection we found them to contain nucleated red cells corresponding in size to those of fowls. In these two important species we also found nucleated red cells which correspond in size to those of geckoes. *P. papatasi* both wild and laboratory bred from Baghdad and Palestine fed readily on geckoes in captivity. As in mosquitos, there are apparently differences in feeding habits in the same species in various localities, for Knowles (1928) states that in Calcutta *P. papatasi* does not feed on lizards. It is evident that the interpretation of infection rates of *Leishmania* in sandflies is difficult, particularly so as morphological characters in *Leishmanias* are of limited or no specific value. All *Herpetomonad* flagellates found in wild sandflies can be regarded as belonging to the genus *Leishmania* (sensu Wenyon) and not to the genus *Herpetomonas*, for owing to the life-history of the sandfly infection from adult insect to larva can only take place by means of resistant cysts capable of living in the ground; this is theoretically possible, but although large numbers of wild and laboratory bred sandflies have been dissected by different observers in various parts of the world, no cysts of *Herpetomonas* have yet been observed and this in spite of the fact that the methods adopted for breeding sandflies are ideal for the propagation of *Herpetomonas*.

The problem is to determine the species of a *Leishmania* found in a wild sandfly. The ideal method is to inoculate *Herpetomonad* flagellates found to adopt an anterior position in a wild sandfly into man, but this is not always practical although we adopted this method in Jericho and Baghdad. Here again a negative result cannot be interpreted with any degree of certainty for it may be due to a natural immunity in the experimental volunteer, to the flagellates not having reached the infective stage or to the fact that the flagellate is not a *Leishmania* of man. On the other hand a positive result is conclusive.

There is one important factor which we could not investigate, namely the seasonal variation, if any, in the infection rate of wild sandflies. Endemic foci of the second type such as we have in

Palestine are not favourable for such investigations for the infection rate is very low ; but an investigation of this type carried out systematically throughout the whole sandfly season in a big endemic centre such as Baghdad should give valuable results. The season in which the majority of cases of oriental sore are acquired is not known because the incubation period varies enormously (from two weeks to three years) and because the early stages of the disease are often overlooked. A determination of the season of maximum infection rate in wild sandflies is necessary in order to establish the period when the disease is most frequently transmitted in nature. Information of this kind would also give indications for prophylaxis.

Jericho. In 1925 the infection rate in *P. papatasi* was found to be about 1 per 1,000. Three wild sandflies were proved to be infected with *L. tropica*, for inoculation of flagellates found in their alimentary tract into three human beings produced lesions in which *L. donovan* bodies were found.

Baghdad. Of 528 wild *P. papatasi* dissected none were infected. Of 683 wild *P. sergenti* two were infected. In one specimen from Haidar Khan there was a slight infection in the stomach and cardia ; in another from Baghdad West the infection in stomach and cardia was very heavy. Flagellates from the latter were inoculated into a human being (27.5.28) with a negative result up to date. The only sources from which *P. sergenti* can acquire an infection with *Leishmania* in Baghdad are as far as our knowledge goes, man, dog and geckoes. The human and the dog's strains of cutaneous Leishmaniasis are probably identical but conclusive proof is still lacking.

Aleppo. No *Leishmania* was found among the 690 sandflies dissected. Wenyon (1912) found a 6 per cent. infection rate among the sandflies of Aleppo. It is interesting to note that according to the statements of local medical practitioners oriental sore is on the decline in Aleppo. Whereas before the war nobody escaped the disease many of the Europeans who came to Aleppo since the war have not become infected. Nevertheless oriental sore is exceedingly common ; nearly all native adults show scars and very many native children are seen with active sores.

We have to thank Dr. E. Libman of New York whose generosity enabled us to carry out this work.

SUMMARY AND CONCLUSIONS

The distribution of sandflies and Leishmaniasis was studied in Palestine, Mesopotamia and Syria.

The diagnostic characters of the sandflies found are given.

Phlebotomus chinensis was found in Palestine and Syria.

The epidemiological in addition to experimental evidence show that *P. papatasi* and *P. sergenti* are carriers of oriental sore.

In Baghdad *P. sergenti* is the main carrier of oriental sore. In Jericho and Bar Elias *P. papatasi* is the only carrier, in Aleppo both species probably transmit the disease.

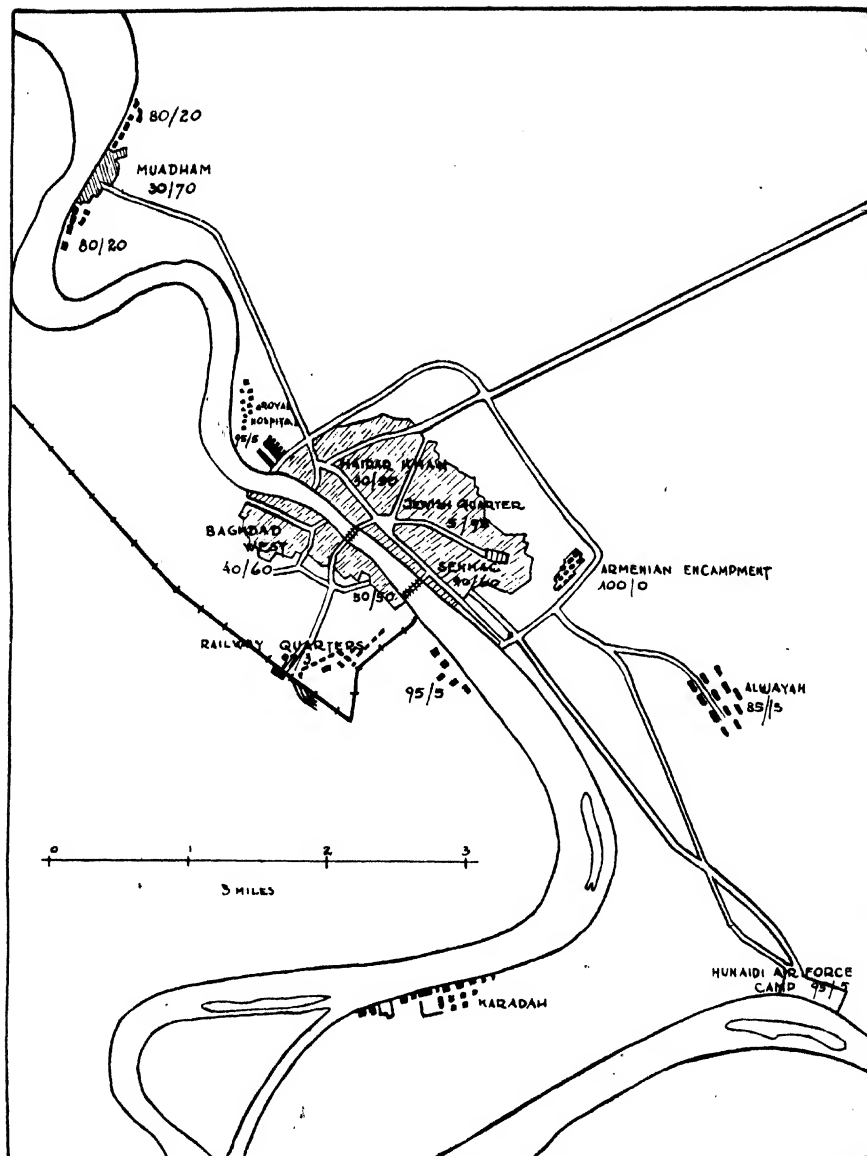
There are localities free from oriental sore in which *P. sergenti* and *P. papatasi* occur.

The peculiarities of the distribution of oriental sore depend on the variations in infectivity of different strains of *L. tropica* for the sandfly vector.

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MAP I. SHOWING THE RELATIVE NUMBERS OF *P. papatasi* AND *P. sergenti* IN BAGHDAD AND SURROUNDINGS.

The first figure gives the percentage of *P. papatasi*, the second of *P. sergenti*, e.g., 40/60 means 40% of *P. papatasi* and 60% of *P. sergenti*.

EXPLANATION OF PLATE IV

- Pharynx of
1. *Phlebotomus papatasi* (female).
 2. *Phlebotomus sergenti* (female).
 3. *Phlebotomus sergenti* (male) collected from two foci.
 4. *Phlebotomus major* (female).
 5. *Phlebotomus chinensis* (female) from Aleppo.
 6. *Phlebotomus chinensis* (female) from Rosh Pinah.

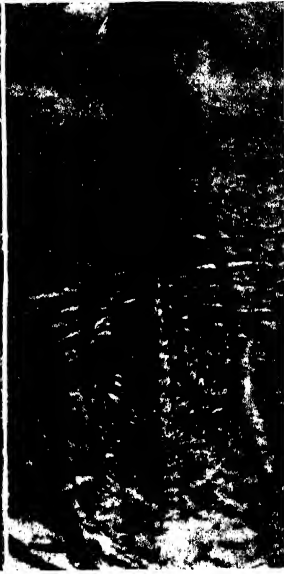
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BLOOD SUGAR IN INFECTIONS WITH *TRYPANOSOMA LEWISI*

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The purpose of the work reported here has been to study the effect upon rat's blood sugar of infections with *Trypanosoma lewisi*. In very recent years several papers have appeared dealing with this subject, but their chief emphasis has been upon the relationship between blood sugar and fatal trypanosome infections. Hence it seemed of interest to test the action upon the glucose level of *T. lewisi*, which, as is well known, produces an infection ending in recovery and complete disappearance of the organisms from the animal's body.

Schern (1925) reported the results of experiments with trypanosomes which tended to show that as the number of organisms in the blood increased, the amount of glucose decreased, and that the terminal symptoms and death of the animal were due to a hypoglycaemia. He believes accordingly that fatal trypanosomiasis, where death occurs with huge numbers of parasites in the blood stream, is a 'sugar sickness.' In a later paper (1928) he reports actual determinations of the blood sugar in rabbits and a horse infected with *T. equinum* and concludes as before that the infection produces a hypoglycæmia, and that death is thus only indirectly due to the invading organisms.

Savino (1927) has reported the effect of infections with *T. equiperdum* and *T. equinum* on the blood glucose in dogs. He showed that the number of trypanosomes varied with the concentration of glucose, being less after the injection of insulin and greater after glucose was given intravenously. He further noted that the spleen plays some part in this variation since after splenectomy neither insulin nor glucose affected the number of trypanosomes.

A similar result was obtained after the nerves leading to the spleen had been severed.

Two other papers should be noted in this brief review. Bruynoghe, Dubois, and Bourkaert (1927) confirmed Schern's work. They used guinea-pigs and rabbits infected with *T. brucei*. They do not believe that the trypanosomes consume the blood sugar directly, but rather that the titer of the sugar is reduced by an insulin-like substance. This substance, they believe, is formed either immediately by the trypanosomes, or by the action of the parasites upon the pancreas. The evidence for this conclusion requires considerable broadening, however, before it can be considered as more than a suggestion.

von Fenyvessy (1926) studied the blood sugar of rats infected with *T. equiperdum*. He discovered that the blood sugar was lowered as the infection progressed and that if the animal were cured by Germanin the level of the glucose returned to normal. He believes with Schern that disturbances in sugar metabolism play a considerable rôle in the pathogenic action of the trypanosomes.

TECHNIQUE

The strain of *T. lewisi* used in this work was kindly furnished by Professor W. H. Taliaferro of the University of Chicago. Blood sugar determinations were made by a new 'micro' method recently described by Folin (1928). This method requires the use of only 1/10 c.c. of blood. The blood itself was obtained by heart puncture under light anaesthesia with iso-amyl-ethyl barbituric acid (amytal), approximately 7 mg. being given per 100 gr. of rat*. The rats were on an ordinary laboratory diet; they were not fed on the day when the test was made.

Three groups of animals were used: normal rats, infected rats, and splenectomized infected rats. These groups will be considered in order.

The first group consisted of thirty-six normal rats. Blood sugar determinations upon them gave an average of 96 mg. of glucose per 100 c.c. of blood. The mean of the observations was 98 mg.

* The effect of iso-amyl-ethyl barbituric acid upon blood sugar concentration is still a matter of experiment. This point does not come into consideration here, however, since this compound was used equally in all the experiments.

and the extremes were 60 mg. and 165 mg. Approximately 90 per cent. of the observations gave values between 70 mg. and 140 mg. of glucose per 100 c.c.

The second series of experiments dealt with rats infected intraperitoneally with a suspension of *T. lewisi* in normal salt solution. The infection was followed by making total counts daily, or on alternate days, of the trypanosomes in the peripheral circulation. When this number had reached its peak, as shown by the increasing uniformity in size of the parasites, the animals were given amytal in the peritoneal cavity and a small amount of blood withdrawn from the heart. The results of several experiments are shown in Table I. The last column in this table gives the blood sugar determination on the same animals taken thirty-five to forty days after infection, when the rats were considered to be free of the infection,

TABLE I.

Blood sugar determinations on rats infected with *Trypanosoma lewisi*.

Rat number	Day after infection	Number of trypanosomes per c.mm. of blood	Blood sugar in mg. per 100 c.c.	Blood sugar in mg. per 100 c.c., 35-40 days after infection
1	8	240,000	136	142
2	8	218,000	139	112
3	8	300,000	132	...
4	9	30,000	151	...
5	9	24,000	126	...
6	9	7,500	136	...
7	9	2,000	126	...
8	9	59,000	113	93
9	7	2,000	117	107
10	7	178,000	83	...
11	8	59,000	117	113
12	8	42,000	102	116
13	7	200,000	78	130
14	7	200,000	89	105
15	6	248,000	95	87

or to have a negligible number of parasites in the blood. The average blood sugar at the height of the infection was 116 gm. the mean 115 mg., and the extremes 78 mg. and 151 mg. No significant variations from the previous determinations were found after the infection had disappeared.

Besides the animals shown in Table I, twelve other rats were used in this part of the work. No actual counts of trypanosomes were made on them, but by means of stained slides it was estimated when the infection was at its height, and the glucose then determined. The average blood sugar in these animals was 115 mg., the mean 111 mg., and the extremes 72 mg. and 154 mg.

The third group was composed of rats which had been splenectomized, in an attempt to increase the severity of the infection. As is well known from the work of Regendanz and Kikuth (1927), the spleen plays a large part in causing *T. lewisi* to cease reproduction and thus is responsible for the fact that this infection is a comparatively transitory one. A considerable difficulty was encountered in this part of the work, however, in that, following splenectomy, many of the rats developed a severe anaemia and died. This anaemia, as Lauda (1925) and Mayer, Borchardt and Kikuth (1926) have shown, is due to *Bartonella muris*. Determinations made on rats thus infected, but without *T. lewisi* infections, showed, however, that the blood sugar did not vary from the normal limits until the agonal stage, when a hypoglycaemia developed. It was, therefore, considered that an influence of *Bartonella muris* infections on the blood sugar could be safely ruled out, especially where, as in the case of the rats shown in Table II no symptoms of *Bartonella* infection were present. It was necessary to consider this point, since Noguchi (1928) has shown that some increase in *Bartonella muris* follows splenectomy in rats, even where a fatal infection is not produced.

As Table II shows, splenectomy did give rise to higher trypanosome counts in the peripheral blood. Not all the animals thus treated, however, gave such high counts, individual animals apparently vary greatly in the severity of their infections. The average blood sugar here was 106 mg. of glucose per 100 c.c. of blood. The mean of the observations was 107 mg. and the extremes 83 mg. and 134 mg.

TABLE II.

Blood sugar determinations on splenectomized rats, infected with *Trypanosoma lewisi*.

Rat number	Day after infection	Number of trypanosomes per c.mm. of blood	Blood sugar in mg. per 100 c.c.
1	10	209,000	99
2	10	470,000	134
3	9	313,000	114
4	7	317,000	113
5	7	253,000	112
6	5	458,000	102
7	6	469,000	83
8	7	311,000	90

DISCUSSION

Infections with *T. lewisi* do not cause abnormal changes in the glucose content of the blood of rats. All the determinations under these conditions fell within the normal range of variation. The slightly higher averages for infected than for normal animals are probably without significance as they lie well within the range of experimental error.

As already stated, Schern and von Fenyvessy attribute a considerable rôle in trypanosome pathogenicity to the gradual lowering of the blood sugar which these organisms cause. Since no experimenters have succeeded in proving the existence of a trypanosome toxin or other antagonistic substance, it is probable that many of the symptoms may in fact be due to a hypoglycæmia. In this event, the absence of symptoms in *T. lewisi* infections would be due to the ability of the experimental animal to maintain a constant level of blood sugar, until the spleen gives rise to the 'reproduction inhibiting' antibody (Taliaferro), and thus ends the severe phase of the infection.

When the results of the present paper were in manuscript, it was found that Regendanz and Tropp (1927) had already published

findings, dealing in part with the same subject. Their results correspond in general to those given here. They believe that death in trypanosome infections is due to a toxin, but only literary evidence is given for this view. The death of infected rats following splenectomy they attribute to extensive proliferation of *T. lewisi*. This conclusion cannot be considered established since they made no actual trypanosome counts, nor did they take into account the possibility of *Bartonella muris* infections in these rats. In our own work, we found that between one-third and one-half of splenectomized rats died of *Bartonella muris*, which corresponds to the number of rats dying in their experiments from *T. lewisi*. Furthermore, the terminal hypoglycæmia which they found in rats dying presumably from *T. lewisi* is also present, as we have shown, in rats dying from *Bartonella muris*. In general the existence of fatal *T. lewisi* infections cannot be accepted, unless the possibility of coincident infection with the highly fatal *Bartonella muris* can be ruled out. A critique of the work of Regendanz and Tropp has recently been published by Schern (1929).

CONCLUSION

The blood sugar of rats is not affected by infections with *T. lewisi*. A hypoglycæmia develops, however, during the terminal stage of infection with *Bartonella muris*, induced by splenectomy.

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The following courses of instruction are given by the Liverpool School of Tropical Medicine each year :—

- (1) Two courses for the Diploma in Tropical Medicine, commencing on the 1st October and the 7th January. The D.T.M. examinations are held in December and March.
- (2) Two courses for the Diploma in Tropical Hygiene, commencing on the 10th January and the 24th April. The D.T.H. examinations are held in March and July.
- (3) Two courses in Veterinary Parasitology, commencing on 1st October and the 7th January.

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This Diploma can only be taken by those who have already obtained the D.T.M.

‘ The course for this Diploma will not be conducted, unless at least five applications are received, and no application for admission can be considered later than December 21st and March 31st respectively.’

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SUSCEPTIBILITY AND RESISTANCE TO TRYPANOSOME INFECTION

VI.—THE COURSE OF THE INFECTION IN SPLENECTOMIZED RATS

BY

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(Received for publication 2 May, 1929)

In the preceding paper (1929) it was pointed out that the rat offered a type of resistance similar in kind, but different in degree from that observed in the guinea-pig. It was also indicated that this resistance was probably referable to the reticulo-endothelial system. It seemed of interest, therefore, to observe the course of infection in splenectomized rats. It was apparent that if the contention was correct, splenectomized rats should react in the same manner as the mouse, that is, the rate of multiplication of the trypanosomes should be uniform and follow a line of geometric progression.

There was a serious difficulty in carrying out this experiment. The splenectomized rats developed an acute Bartonella infection and succumbed in five or six days, before the trypanosome infection had run its course.

This difficulty was eliminated by the observation made by Meyer (1927) that an injection of salvarsan prior to the removal of the spleen prevented the appearance of Bartonella for some time. Using this procedure it was possible to observe the course of infection in splenectomized rats without interference of other factors.

The results of these experiments constitute the subject of this paper.

EXPERIMENTAL

SERIES I. Large rats, 120 to 150 grams in weight, were used. These were divided into three groups. One set received 0.5 c.c. of a 1:1,000 solution of Neosalvarsan intraperitoneally and the spleen was removed two or three days later. A second set was splenectomized without previous treatment with salvarsan. The third set served as a control.

Two sets of experiments were carried out, identical in all essentials, except that in one a small dose of trypanosomes was injected subcutaneously, while in the other a somewhat larger dose was inoculated intraperitoneally. In a number of rats in each experiment, red cell and trypanosome counts were made daily. In the others only the incubation period and the end results were noted. *T. evansi* was used in all experiments.

The results were striking and consistent. The untreated splenectomized rats developed a Bartonella infection and in most cases died with a severe anaemia either before the trypanosome infection had developed or during its progress. In the splenectomized animals treated with neosalvarsan neither the Bartonella infection nor anaemia developed. On the contrary, the anaemia, which is usually associated with a trypanosome infection, was not observed in the salvarsan treated animals.

In all of the animals the course of the infection differed decidedly with the method of inoculation. The control animals differed, however, in this respect from the splenectomized ones. In the former the course of infection was more rapid when the infection was by the subcutaneous route, while in the latter the reverse was the case.

The results of these experiments are presented below (p. 317).

It will be noted that there was practically no difference in the incubation period and duration of illness between the salvarsan-treated splenectomized rats and the control. Nor was there any difference in the course of the infection. The only striking difference is that in the salvarsan-treated animals the red cell count at death was nearly 8,000,000 (the normal count), while in the control group it was reduced to 5,000,000.

Quite a different result was obtained when the infection was given by the intraperitoneal route.

TABLE IA.

Effect of splenectomy on a trypanosome infection in rats. (Infected subcutaneously.)

Number of rats	Average weight	Mode of infection	Dose	Splenectomized	Salvarsan	Average incubation	Average duration of illness	Average tryp. count at death	Average red cell count at death
5	142	Subcut.	10,000	+	+	6.4	13.4	1,860,000	7,975,000
5	136	"	10,000	+	-	*6.0	...	*900,000	2,300,000
5	137	"	10,000	Control		6.0	14.0	1,700,000	5,100,000

* Only one animal survived, all the others died five to six days after the spleen was removed, with a red cell count of 1,800,000; the remaining animal died before the trypanosome infection had run its course.

TABLE IB.

Effect of splenectomy on a trypanosome infection in rats. (Infected intraperitoneally.)

Number of rats	Average weight	Mode of infection	Dose	Splenectomized	Salvarsan	Average incubation	Average duration of illness	Average tryp. count at death	Average red cell count at death
4	141	Intra-per.	65,000	+	+	3 d.	7½	1,900,000	6,200,000
5*	136	"	65,000	+	-	3	*...	*500,000	1,650,000
4	124	"	65,000	Control		5	15	1,800,000	4,600,000

* Two died before the onset and three in the middle of the infection.

In this series the course of the infection in the splenectomized rats was strikingly different from that in the control. The incubation period as well as the duration of the infection was only half as long as in the control group. Again, the red cell count at death in the salvarsan-treated animals was relatively high as compared with that of the control untreated animals. Typical detail data of the course of infection in the two series are shown in Tables II and IIA.

TABLE II.

Course of infection in splenectomized and control rats infected intraperitoneally with same dose of *T. evansi*.

(Infected 17 January.)

Date	Splenectomized + salvarsan		Control (no salvarsan)	
	142 grm.	145 grm.	126 grm.	122 grm.
Jan. 20	+ in drop	+ in drop	+ in drop	+ in drop
Jan. 21	4,000	2,000	- in drop	- in drop
Jan. 22	42,000	44,000	2,000	- in drop
Jan. 23	438,000	478,000	2,000	+ in drop
Jan. 24				
a.m.	1,200,000	1,900,000	...	+ in. drop
p.m.	1,600,000	2,200,000
	died 24-25	died 24-25		
Jan. 25	16,000	2,000
Jan. 27	82,000	2,000
Jan. 28	100,000	10,000
Jan. 29	700,000	...
Jan. 30	1,800,000	250,000
			died 30-31	
Jan. 31	700,000
Feb. 1	900,000
				died 1-2

TABLE IIa.

Same as above, infection subcutaneous with same dose of *T. evansi*.

(Infected 14 January.)

Date	Splenectomized + salvarsan		Control (no salvarsan)	
	145 grm.	145 grm.	136 grm.	145 grm.
Jan. 20	—	—	...	+ in drop
Jan. 21	—	—	+ in drop	—
Jan. 22	2,000	8,000	+ in drop	...
Jan. 23	12,000	6,000	10,000	32,000
Jan. 24	4,000	—	4,000	20,000
Jan. 25	6,000	20,000	+ 4,000	12,000
Jan. 26
Jan. 27	400,000	220,000	160,000	330,000
Jan. 28				
a.m.	950,000	1,300,000	130,000	350,000
p.m.	1,600,000	1,400,000
	died 28-29	died 6 p.m.		
Jan. 29	300,000	1,600,000
Jan. 30	1,650,000	1,670,000
a.m.		died 10 a.m.
p.m.	1,800,000	...
			died 30-31	

Summarising these experiments it appears that (1) Splenectomized animals treated with salvarsan and infected by the subcutaneous route did not show any decreased resistance as compared with the corresponding untreated control group; (2) However, animals so treated and infected by the intraperitoneal route manifested a striking reduction in resistance as compared with the corresponding control group, the course of the infection being the same as that observed by Doerr and Berger (1922) in mice; (3) The salvarsan not only prevented the development of Bartonella infection for over two weeks, but it seemed also to protect the red cells against the destructive effects of the trypanosome infection; (4) Death in trypanosome infections was apparently not affected by the absence of anaemia.

SERIES 2. These experiments were repeated with the same results. The following experiment is illustrative. The procedure was the same as above, except that the same doses were given intraperitoneally and subcutaneously, and all the animals were inoculated at the same time with the same material. The data of this experiment are summarised in Tables III and IV.

TABLE III.

Comparison of the duration of infection in splenectomized, salvarsan-treated and normal rats.

Treatment	Mode of inoculation	Dose	Average weight	Average incubation. Days	Average duration of illness. Days	Final tryp. count; average	Initial red cell count; average	Final red cell count; average
0.3 c.c., 1 : 1,000 sol. neo-salvarsan i.p., 5.ii.28; Splenectomy, 6.ii.28; Infection, 7.ii.28	Subcutaneous	25,000	26.5	4	11½	1,850,000	4,300,000	3,900,000
Salvarsan as above, 5.ii.28; Spleen not removed. Infected, 7.ii.28.	"	25,000	26.5	6	18½	1,800,000	4,500,000	4,700,000
Untreated Control; Infected, 7.ii.28.	"	25,000	24.0	5	10½	1,850,000	4,250,000	3,250,000
0.3 c.c., 1 : 1,000 sol. neo-salvarsan i.p., 5.ii.28; Splenectomy, 6.ii.28; Infection, 7.ii.28.	Intra-perit.	25,000	26.0	3	8	1,450,000	4,550,000	3,100,000
Salvarsan as above, 5.ii.28; Spleen not removed; Infected, 7.ii.28.	"	25,000	27.5	3	14½	1,800,000	5,000,000	3,750,000
Untreated Control; Infected, 7.ii.28.	"	25,000	22.0	4	13½	2,200,000	4,800,000	3,200,000
0.3 c.c., 1 : 1,000 sol. neo-salvarsan; Spleen removed, 6.ii.28; Not infected.	4,700,000	6,100,000 (21.ii.28)

TABLE IV.

Median course of infection in splenectomized, salvarsan treated and normal rats.

(Infected February 7, a.m.)

Date	Daily trypanosome count—per c. mm.					
	Splenectomized rats treated with salvarsan		Control rats		Salvarsan treated normal rats	
	Intra-peritoneally	Sub-cutaneously	Intra-peritoneally	Sub-cutaneously	Intra-peritoneally	Sub-cutaneously
Feb. 10	+ in drop	+ in drop	...
Feb. 12	8,000	+ in drop	+ in drop	+ in drop	4,000	...
Feb. 13	92,000	+ in drop	+ in drop	—	—	+ in drop
Feb. 14	466,000	+ in drop	6,000	34,000	2,000	2,000
Feb. 15	1,000,000 died 1 p.m.	2,000	4,000	80,000	2,000	8,000
Feb. 16	460,000	...	26,000
Feb. 17	...	80,000	8,000	700,000	4,000	16,000
Feb. 18	...	160,000	16,000	2,500,000	8,000	30,000
Feb. 19	...	750,000	44,000	...	18,000	24,000
Feb. 20	...	1,200,000 died 10 p.m.	300,000	...	22,000	42,000
Feb. 21	650,000	...	112,000	150,000
Feb. 22	1,800,000 died 2 p.m.	...	1,000,000 died 12 p.m.	350,000 died 25.ii, 2 p.m.

NOTE.—The counts were all made at the same time—between 8 and 9 a.m.

It is clear from these tables that splenectomized rats infected by the intraperitoneal route succumbed long before those infected subcutaneously or the corresponding controls. In normal rats the mode of inoculation also materially affected the course of the infection, but in an opposite manner: those infected by the subcutaneous route succumbed sooner than those receiving the same dose intraperitoneally. Another important point brought out in these experiments is that the salvarsan injection, as such, modified the course of infection in normal animals, but that this effect was either not apparent (intraperitoneal infection) or greatly reduced (subcutaneous infection) in the splenectomized rats.

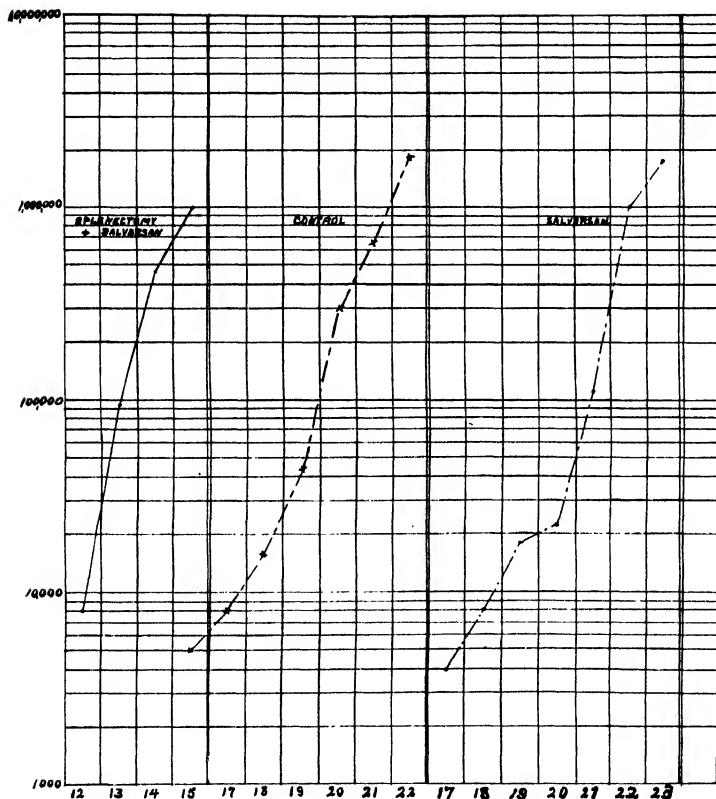


CHART I. Course of infection in rats treated as indicated and inoculated intraperitoneally. (The animals were of the same weight and received the same dose of *T. evansi* 7.ii.28.)

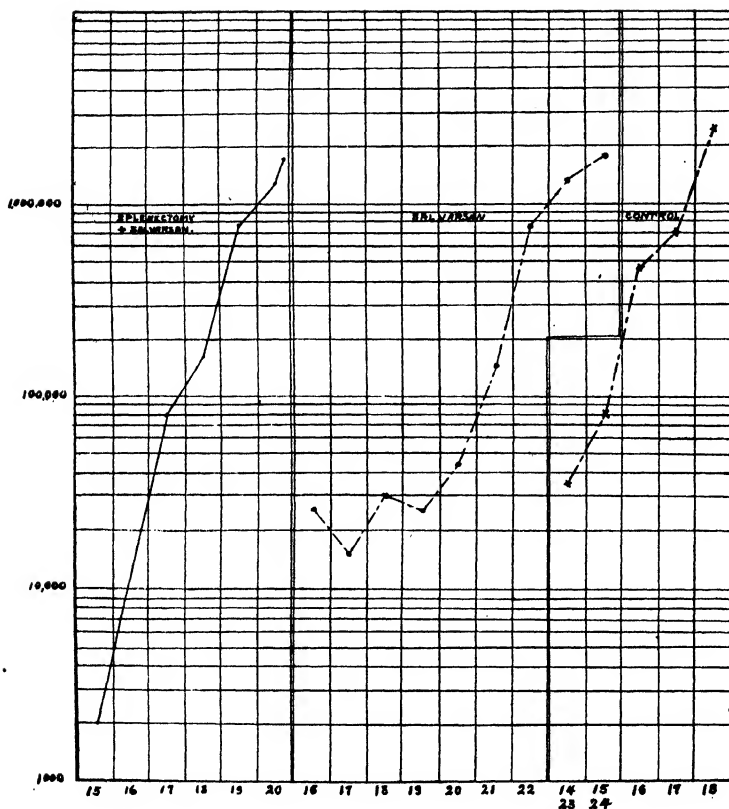


CHART II. Course of infection in rats treated as indicated and inoculated *subcutaneously*. (The animals were of the same weight and received the same dose of *T. evansi* 7.ii.28)

The detailed course of the infection in splenectomized and non-splenectomized animals infected by various routes is shown in Table IV and Charts I and II. It is apparent that in splenectomized animals inoculated intraperitoneally the native resistance is almost completely broken down despite the salvarsan. Both the generation time and the curve of multiplication of the trypanosomes in the peripheral circulation correspond with that usually observed in mice.

DISCUSSION

The experiments reported above bring to light a number of significant facts. It appears that in normal rats the resistance to a trypanosome infection is highly localised, the chief organ of defence being the spleen. Normal animals infected subcutaneously succumb much sooner than those inoculated intraperitoneally. In salvarsan-treated animals the opposite is the case. But, when salvarsan-treated rats are splenectomized, then the results are again reversed. Despite the salvarsan, the animals infected intraperitoneally lose all resistance to infection; at the same time, the resistance to a subcutaneous infection seems to be greater than that of untreated controls.

This peculiar behaviour of salvarsan is particularly interesting in relation to the mechanism of chemotherapy. Krichewski and Meersohn (1926) first showed that the reticulo-endothelial system, particularly the spleen, plays a definite part in the therapeutic effect of salvarsan. Subsequently, Feldt and Schott (1927), Jungblut (1927), as well as Kritchewski (1927) showed that this is also true of other drugs. Kritchewski (1927, 1928) expressed the view that the spleen acts merely as a carrier of the drug, storing it up and then slowly liberating it. The heightened resistance to the subcutaneous infection in comparison with normal controls and the suppression of the Bartonella would indicate that even in splenectomized animals the salvarsan is stored and is active despite the removal of the spleen. Its activity is entirely absent only in splenectomized animals infected intraperitoneally. But this merely emphasizes the importance of the spleen as an active participant in the therapeutic action of the drug. Evidently other parts of the reticulo-endothelial system also play a part.

Another interesting point is the apparently protective effect of salvarsan on the red cells. Anaemia is a characteristic element in the pathology of a trypanosome infection. In the above experiments the salvarsan-treated animals did not show the typical picture. On the contrary, there appeared to be a stimulation of the red cells. It would seem possible that the prevention of the Bartonella may be due to this protective action of the drug on the red cells. This possibility is supported by the fact that as soon as the salvarsan effect disappears—usually about three weeks—the Bartonella appear and the animals succumb in five or six days.

CONCLUSIONS

1. Salvarsan increases the resistance of normal rats to a trypanosome infection and appears to exert a protective effect on the red blood cells. The former effect is either absent or greatly reduced in splenectomized rats.

2. Splenectomized rats treated with neosalvarsan to prevent a Bartonella infection, show a complete absence of resistance to a trypanosome infection when the inoculation is given intraperitoneally. The course of infection in such rats follows a simple curve of geometric progression.

3. No corresponding reduction in resistance is noted in such rats when the inoculation is given subcutaneously. This is apparently attributable to the action of the salvarsan.

4. In normal rats the course of the infection is more rapid if the inoculum is given subcutaneously than it is when given intraperitoneally.

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SUSCEPTIBILITY AND RESISTANCE TO TRYPANOSOME INFECTIONS*

VII.—CAUSE OF INJURY AND DEATH IN TRYPANOSOME INFECTED RATS

BY

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(Received for publication 6 May, 1929)

The cause of death in experimental trypanosome infections has been a subject of considerable discussion. The infection in the rat is relatively so simple that many investigators have turned their attention to a study of the course of a trypanosome infection in this animal in the hope of elucidating the mechanism of injury and death in trypanosome and allied infections.

Two opposing views have evolved. Schilling and Rondoni (1913), Martin and Darré (1914), Reichenow (1921), and more recently, Regendanz and Tropp (1927) assume that the injury and ultimate death are due to a toxic substance liberated by the disintegration of the parasites. Reichenow finds support for this view in the fact that in man the height of the temperature is reached after the trypanosomes disappear from the circulation.

On the other hand, Schern (1926, 1928) and Fenyvessy (1926) maintain that the cause of injury and ultimate death is to be found in the exhaustion of the blood sugar and glycogen reserve. These authors found that not only do trypanosomes utilize sugar *in vitro*,

* A preliminary report of a part of this work was published by Kligler, I. J., and Geiger, A. (1928), in the *Proc. of the Soc. Exp. Biol. and Medicine*, 26, 229.

but that a definite hypoglykemia develops in the infected animal in the course of the infection. Regendanz and Tropp (1927), who support the toxin theory, were able to confirm the findings of Schern and Fenyvessy, but their results indicate that the glycogen depletion is by no means complete and that at the height of the infection sufficient glycogen remains in the liver to maintain a normal sugar concentration in the blood. They maintain that the decrease in the blood sugar is due to a depressive effect on sugar inversion by the trypanosome toxin and not to the depletion of glycogen.

In further extension of his views, Fenyvessy (1927) studied the respiratory exchange of infected animals and published data which indicate a more active metabolism in trypanosome infected animals than in normal ones, particularly towards the end of the infection. His data show that the oxygen consumption of infected animals increases with the increase in the number of trypanosomes in the blood, and he concluded that this increase is due directly to the metabolism of the trypanosomes. Scheff (1928), in continuation of Fenyvessy's work, found further that there is a greater utilization of the blood oxygen in trypanosome infected than in normal rats, and a progressive decrease in the oxygen saturation of the blood.

It is clear, therefore, that there exist two opposing views. One ascribes the changes to the action of a toxin, the other to the direct injury due to the sugar depletion by the metabolic activity of the trypanosomes. Neither view is adequately supported by experimental facts. There is also a discrepancy in the analytical data reported by Regendanz and Tropp (1927) and those by Schern (1928) and Scheff (1928). The data presented by the former show that there is only a partial glycogen exhaustion, while the latter claim that the exhaustion is complete. At the same time, Scheff (1928) states that he is unable to account for the fact that the cure of a heavily infected animal with 'Bayer' or other drug leads to a prompt recovery of the blood sugar concentration to normal.

One aspect of the problem seems to have escaped the previous workers. Although there is general agreement that the blood sugar concentration is depressed, no attention has been given to the possible harmful effect of intermediate products of the sugar metabolism. It seemed to us more than likely that the depression of the sugar concentration in the blood was due to its active utilization

by the trypanosomes, so active that readjustment could not keep pace; and that this active glucose metabolism led to a state of constant high lactic acid concentration in the blood. This view would account also for the prompt equalization of the blood sugar after the trypanosomes had been eliminated by a drug or otherwise. We, therefore, decided to determine the lactic acid concentration and alkali reserve in the blood at various stages of the infection. In this paper we deal specifically with the lactic acid concentration.

EXPERIMENTAL

Preliminary to the main object of this investigation, we attempted to test the opposing views in a direct manner. We also attempted to repeat Fenyvessy's metabolism experiments.

Toxic Effect of Trypanosomes. Massive doses of trypanosomes were injected repeatedly into rats. No ill-effects were noted. The same results were obtained by injection of serum obtained from infected rats shortly before death. The following protocols are typical of several experiments with the same results.

A heavily-infected guinea-pig was bled into citrate solution. The blood was sedimented at low speed, to throw down the red cells. The supernatant fluid was sedimented at high speed and the sedimented trypanosomes separated from the clear fluid. The sediment was re-suspended in a small amount of distilled water and stored in the ice-box. The supernatant serum was also stored.

The trypanosomes and plasma respectively were injected repeatedly into a series of rabbits and rats. Control animals were given saline injection. The experiments were repeated with *T. evansi*, *T. gambiense*, and *T. rhodesiense*.

The *T. gambiense* treated animals were given seven injections, the others received ten injections, in the course of two weeks. In the rabbits the injection of the autolized trypanosomes and, to some extent, also the plasma, was associated with a leucopenia. No other toxic manifestation was observed either in the rats or rabbits. On subsequent infection of the rats with the homologous strain, the treated animals died at the same time or a day or two before the controls.

It seems, therefore, that even the repeated injection of heavy doses of trypanosomes does not exert an appreciable toxic effect on the animal. At any rate, the toxic effect is not sufficient to account for the severe damage and ultimate death caused by the infection.

The Effect of Supplementary Injections of Glucose. If carbohydrate depletion is the direct cause of injury and death, then daily injections of glucose should at least modify the course of the illness. A series of experiments were made to test this assumption. The results are shown in the following table.

TABLE I.

Effect of glucose injections into rats on the course of a Trypanosome infection.

Number of rats	Weight in grms., average	Dose	Date infected	Incubation time, days	Duration of infection, days	Treatment
2	53.5	150,000	3.vi.28	5	17.5	Untreated
2	53.5	150,000	3.vi.28	5	19.5	0.5 c.c. 10% glucose sol. from 31.v.28 to end
5	33.2	10,000	22.vi.28	11	15.0	Untreated
6	33.3	10,000	22.vi.28	10	15.0	0.5 c.c. 10% glucose sol. daily from 4.vii.28 to end
6	33.2	50,000	28.xi.28	3.5	8.5	Untreated
6	33.2	50,000	28.xi.28	3.0	8.7	0.5 c.c. 10% sol. of glucose daily from 1.xii.28 to end
5	33.4	50,000	13.xi.28	5.8	16.0	Untreated
5	33.6	50,000	13.xi.28	6.0	13.0	0.5 c.c. 10% glucose daily from 19.xi.28 to end
6	36.3	16,000	25.i.29	4.0	17.8	Untreated
6	41.5	16,000	25.i.29	4.5	22.8	0.5 c.c. glucose injection twice daily, and 1.0 glucose supplementary to diet; beginning 2 days after the infection to the end

The results indicated an advantage when the glucose treatment was begun before or at the beginning of the infection. The groups which were given glucose from the time that trypanosomes first appeared in the circulation, that is at the end of the incubation period, showed no advantage over the untreated group. In the three series in which the glucose injections were begun at the onset of the infection, the average duration of the infection in the untreated rats was 12.9 days, while in the treated ones it was 12.2 days. In the two other series the advantage is definitely with those rats which received glucose—17.7 days against 22 days.

Respiratory Exchange of Trypanosome Infected Animals. The respiratory exchange was studied on a large series of infected rats. To eliminate errors in experimentation the normal animals were first accustomed to the apparatus. At the beginning the animals were extremely restless. After several tests, however, they remained perfectly quiet even for several hours. Observations were first made on normal animals which had become accustomed to the apparatus; these animals were then infected and the respiratory exchange measured at various stages of the infection as determined by direct chamber counts. The infecting dose was small (25,000 Tr.) in order to prolong the duration of the illness.

The apparatus used for the respiration experiments is a modification of the Benedict apparatus devised by Geiger. In several instances the Haldane apparatus was used as a control. The results obtained with the two systems were in entire agreement. The animals remained in the apparatus at least two hours, and each time the apparatus was tested before and after the experiment to assure absence of leakage.

One would assume, on the basis of the observations made by Nauss and Yorke (1911) and others, that trypanosomes consumed oxygen *in vitro*, that *in vivo* as well their activity would be marked by a higher oxygen consumption by the animal. This, however, we failed to observe in many oft-repeated tests. There appears, on the contrary, a depression in activity, the reason for which is not clear; this question will be discussed more fully below.

The results are shown in Table II. It is apparent that there is no increase in oxygen consumption in trypanosome infected rats, as compared with normal ones. On the contrary, there seems

often, in the last stages of the infection, to be a lowered oxygen consumption. It should be noted that we used a species of trypanosome different from that of Fenyvessy. In all our experiments we used the strain of *T. evansi* which had been isolated by us (Kligler and Weitzmann, 1924) about five years ago, from mules.

TABLE II.
Respiratory exchange in trypanosome infected rats.

Number of rat	Day of examination	Weight in grammes	O ₂ consumed per hour per kilo.	Respiratory quotient	Number of trypanosomes per cubic mm. of blood	Date of death
McCollum	Diet—		Old rats			
1	3.iv.28	185	1,377 c.c.	0.81	0	
1	9.iv.28	184	1,383 "	0.80	0	
1	27.iv.28	181	1,381 "	0.78	48,000	1.v.28
1	30.iv.28	181	1,367 "	0.79	1,166,000	
2	3.iv.28	179	1,379 c.c.	0.86	0	
2	15.iv.28	178	1,377 "	0.83	0	
2	27.iv.28	176	1,369 "	0.80	8,000	3.v.28
2	30.iv.28	176	1,350 "	0.77	60,000	
			Young rats.			
3	5.ii.28	96	2,080 c.c.	0.86	0	
3	6.ii.28	96	2,080 "	0.86	0	6.iii.28
3	14.ii.28	102	2,128 "	0.88	0	
3	28.ii.28	102	2,082 "	0.80	Infected	
3	3.iii.28	102	1,904 "	0.82	Heavy infection	
4	7.ii.28	112	1,972 c.c.	0.88	0	
4	27.ii.28	111	1,962 "	0.89	Heavy infection	28.ii.28
4	28.ii.28	111	1,622 "	0.71	Heavy infection 2 hours before death	
5	29.i.28	130	1,873 c.c.	0.83	0	
5	3.ii.28	131	1,943 "	0.80	0	4.iii.28
5	2.iii.28	132	1,940 "	0.77	Heavy infection	
Salt Poor	Diet—		Sodium poor diet			
6	29.iii.28	181	1,490 c.c.	0.71	0	
6	29.iv.28	183	1,458 "	0.74	700,000	1.v.28
6	30.iv.28	182	1,479 "	0.72	1,600,000	
7	22.iv.28	136	1,531 c.c.	0.87	0	30.iv.28
7	29.iv.28	132	1,491 "	0.79	1,034,000	
			Potassium poor diet			
8	30.iii.28	148	1,800 c.c.	0.81	0	
8	1.iv.28	148	1,872 "	0.88	0	29.iv.28
8	29.iv.28	132	1,890 "	0.81	1,034,000	
9	30.iii.28	132	1,580 c.c.	0.81	0	
9	1.iv.28	132	1,642 "	0.84	0	
9	29.iv.28	132	1,621 "	0.79	734,000	

Lactic Acid Production. The negative results obtained in the experiments reported above and more particularly the absence of an increase in the respiratory exchange of infected animals, strengthened our first assumption, that incompletely oxidised metabolic products of glucose, probably lactic acid, resulted from the activity of the trypanosomes and gave rise to an acidosis. We, therefore, directed our attention to the presence of lactic acid in infected animals.

The quantitative determination of lactic acid in the blood is a somewhat complicated procedure, and care must be taken to prevent any convulsive movements on the part of the animals which, in themselves, would cause a rapid rise in the lactic acid content. The procedure adopted was as follows :—The animal was starved twelve hours before being bled. It was then placed under a beaker containing a cotton wad soaked in ether. The animal dozed off slowly with relatively few movements, and in about one minute was in deep narcosis. It was immediately placed on the board, a thin canula inserted in the carotid artery and the blood withdrawn with a glass syringe. After a little practice the whole procedure lasted only a few minutes.

The arterial blood was examined by the method described by Friedmann, Cotonio and Shaffer (1927). Heparin was used to prevent coagulation.

Before the tests were made on infected animals, a series of normal animals were examined by the same procedure. Likewise, each time a normal control was run to check the procedure. The results seem, therefore, relatively free of error.

At the time when blood was taken for lactic acid determination, a red cell count, haemoglobin determination and trypanosome count were made. The lactic acid data could, therefore, be correlated with other findings.

The results are shown in Table III. In the first part of the table are cited data for normal animals. The average red cell count is about 7,000,000, the haemoglobin index over 90, and the lactic content 30 mgm. per 100 c.c. of blood. In the infected animals there is a progressive decrease in the red cell count with the increase in the number of trypanosomes, the haemoglobin index is only slightly reduced, but there is a definite progressive rise in the lactic

acid concentration parallel to the rise in the number of trypanosomes. In instances where the animal is approaching exitus—Nos. 15 and 16—the amount of lactic acid is relatively enormous, three to four times the normal concentration.

TABLE III.

Lactic acid in normal and trypanosome infected blood.

Rate	Number of trypanosomes per c.mm.	Number of red cells per c.mm.	Haemoglobin index ; Sahli	Lactic acid ; mgm. in 100 c.c. blood
1. Normal	...	7,000,000	95	35.0
2. " "	..	7,500,000	98	29.0
3. " "	29.5
4. " "	...	6,800,000	92	27.7
5. " "	...	6,450,000	86	26.0
Average...	30
1. Tryp. Inf.	10,000	8,000,000	...	29.6
2. " "	12,000	6,500,000	85	52.7
3. " "	50,000	7,700,000	85	57.0
4. " "	56,000	6,600,000	83	58.0
5. " "	118,000	7,000,000	78	72.6
6. " "	120,000	7,000,000	86	72.0
7. " "	300,000	6,000,000	78	43.0
8. " "	300,000	30.8
9. " "	668,000	5,800,000	80	48.2
10. " "	1,080,000	...	78	103.0
11. " "	1,180,000	...	82	106.0
12. " "	1,450,000	5,000,000	80	94.0
13. " "	1,600,000	7,000,000	...	87.0
14. " "	1,650,000	5,000,000	...	101.0
15. " "	1,850,000	4,000,000	62	120

It would seem, therefore, that the trypanosome infected animal is constantly in a state of acidosis even when the number of organisms is still relatively small, 50,000 to 100,000 per c.mm. Whether the lactic acid is due directly to the trypanosomes or indirectly to an impairment in the oxidative processes of the tissues, is not clear. In either case, there seems to be an insufficiency in the oxidative mechanism to complete the oxidation of the lactic acid. This is in harmony with the data on the respiratory quotient recorded above.

Nor is the reason for the lowered oxidation quite clear. The decrease in the number of red cells does not account fully for this phenomenon because the red cells are decreased rather slowly at first and only towards the end is there a sharp and sudden diminution in their numbers. Two possibilities present themselves. One is that there is a rapid consumption of oxygen by the trypanosomes, leading to a lowering in the oxygen tension of the blood and depriving the tissues of their needed oxygen. In favour of this view is the drop in the oxygen saturation of the blood in infected animals observed by Scheff (1928). The data reported by this author show, however, considerable variations and our own observations thus far reveal very little difference in the oxygen content of the arterial blood of normal and infected rats.

The other possibility is that the lactic acid is produced continually by the trypanosomes and too rapidly for complete oxidation and that the lactic acid in turn affects the oxidative process. Thus a vicious circle is established. This view is partly supported by the observations made by Geiger (1929) that *in vitro* certain anions, and particularly lactate, deflect the isoelectric point of haemoglobin. This effect is, however, dependent on the concentration of the anion, and it is doubtful whether in the early stages of the infection this constitutes the most important phase. The process is obviously a cumulative one beginning with a rapid and continuous production of lactic acid, leading probably to a progressive depletion of the alkali reserve, a reduced oxidation and a still greater accumulation of lactic acid, resulting in further depletion of the alkali reserve and depression of the normal oxidative processes; death finally resulting from asphyxia.

That the acidosis plays a considerable part in the pathology of the disease is indicated by experiments now under way. Two sets

of five rats of the same weight were infected at the same time with *T. evansi*; one set was untreated and the other received twice daily, 0.5 c.c. 10 per cent. bicarbonate solution intraperitoneally. In spite of the evident injury due to excessive bicarbonate, the average duration of life in the control group was 18 days, and in the bicarbonate treated group, 26.5 days—almost a 50 per cent. increase in the duration of life. These results are very suggestive.

CONCLUSIONS

1. Injection of large doses of trypanosomes, or serum taken when the trypanosome number is at its maximum, does not produce any visible toxic symptoms in rats.

2. Daily injection of glucose to supplement the food affects the course of infection favourably only if started at the time of inoculation and not if started after the incubation period.

3. The oxygen consumption of trypanosome infected rats is not increased; towards the end of the infection it appears somewhat lower than normal.

4. Parallel with the increase in the number of trypanosomes, there is a progressive increase in the concentration of lactic acid in the blood—in the later stages up to three or four times the normal.

5. It is suggested that the pathological processes are engendered by the metabolism of the trypanosomes which results in the rapid production of lactic acid, leading to exhaustion of the alkali reserve and probably also to a depression of the oxidative processes by the specific effect of lactic acid on the haemoglobin.

6. Experiments are under way which indicate that injection of bicarbonate tends to increase the life of the animals as compared with the untreated controls.

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NOTES ON TREATMENT OF FIFTY-TWO CASES OF RHODESIAN TRYPANOSOMIASIS WITH BAYER 205 AND TRYPARSAMIDE

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During the latter half of 1924 an outbreak of human Trypanosomiasis was discovered in the Ufipa District of Tanganyika Territory, and early in the following year cases were also found in the adjacent District of Tabora.

The species of Tsetse present, *G. morsitans*, the acute nature of the disease, a few months' duration only, and the finding of 'posterior-nuclear forms' in a rat inoculated with the Ufipa strain, all indicated that the disease was of the Rhodesian type.

Fifty-two cases (all of whom were diagnosed microscopically) commenced treatment between November, 1924 and the end of 1925, and nearly all the survivors were kept under observation until 1928.

In twenty-one cases injections of 1.0 to 1.4 grammes of Bayer 205 were given on the 1st, 10th, and 28th days respectively; four patients died before the second injection was due.

In three cases weekly injections of Bayer 205 were given.

Three cases were given Tryparsamide in 3-gramme doses weekly for eight weeks.

The method of treatment subsequently adopted and administered to twenty-five cases was to give a minimum of two injections of Bayer 205, each of 1 gramme, followed after one or two months' interval by twelve weekly injections of Tryparsamide. The first two doses of Tryparsamide were usually 2 grammes, and afterwards the dosage was gradually increased to 4 grammes; there was generally an interval of one month between the fourth and fifth, and between the eighth and ninth injections. The drugs were

administered either intramuscularly or intravenously. Children were generally given proportionately smaller doses.

Unfortunately it was difficult to get patients to attend regularly, with the result that a large proportion did not receive treatment at the specified time, and some did not receive the total dosage laid down for them.

For the purposes of analysing the results of treatment the following classification is adopted* :—

CLASS 1. Comprises cases with no appreciable oedema or wasting before treatment commenced. This Class consists almost entirely of cases of less than six weeks duration and of 'carriers,' *i.e.*, infected persons with slight symptoms of insidious onset or no symptoms at all.

CLASS 2. Comprises cases with appreciable oedema and wasting before treatment, but who are still able to walk about and attend to their ordinary wants.

CLASS 3. Comprises cases who are asthenic and emaciated and too ill to attend to their ordinary wants.

The following are typical histories :—

CASE U17. Female. Adult.

Condition before treatment : Ill about three months. Emaciated. Oedema of legs and feet. Just able to walk. Trypanosomes found in the blood.

Treatment (First course) : Bayer 205, three injections intramuscularly in 1·2 to 1·3-gramme doses on 2.12.24, 12.12.24 and 29.12.24.

Total dosage 3·8 grammes.

Result : Apparent recovery for a time, but was again in indifferent health in September, 1925.

Treatment (Second course) : Bayer 205, four injections in 1-gramme doses between 9.9.25 and 7.10.25.

Total dosage 4 grammes.

Result : Though improved, trypanosomes appeared again in the blood in December, 1925, and by February, 1926, there was a definite clinical relapse.

Treatment (Third course) : Bayer 205, three injections in 1-gramme doses from 7.3.26 to 14.3.26.

Total dosage 3 grammes.

Result : Died 22.3.26.

* The cerebro-spinal fluid was not examined before treatment in any of these cases but in the routine examination of other series of cases it has hitherto been found that oedema is practically never present except when the cerebro-spinal fluid is infected.

CASE U20. Female. Age about 30 years.

Condition before treatment : Ill about one month. No emaciation or oedema. Able to walk about for miles. Trypanosomes present in the blood.

Treatment : Bayer 205, three injections intramuscularly in 1·2-gramme doses on 2.12.24, 12.12.24, and 29.12.24.

Total dosage 3·6 grammes.

Result : Uninterrupted recovery. She was quite fit when seen in February, 1928.

CASE T57. Male. Age about 25 years.

Condition before treatment : Ill one month. Emaciated. Oedema of feet. Hardly able to walk. Trypanosomes present in the blood.

Treatment (First course) : Bayer 205, three injections intravenously in 1-gramme doses between 12.9.25 and 27.9.25.

Total dosage 3 grammes.

Treatment (Second course) : Tryparsamide six injections in 3 to 4-gramme doses from 27.10.25 to 18.12.25.

Total dosage 21 grammes.

Treatment (Third course) : Tryparsamide eight injections in 3-gramme doses, from 1.5.26 to 3.7.26.

Total dosage 24 grammes.

Result : Recovery. No relapses. Was fit end of 1927. Not seen, but reported to be fit, end of 1928.

CASE T64. Female. Age about 25 years.

Condition before treatment : Ill three weeks. Emaciated. Oedema legs and feet. Able to walk a little. Trypanosomes present in blood.

Treatment (First course) : Bayer 205, three injections in 1-gramme doses from 7.10.25 to 21.10.25.

Total dosage 3 grammes

Treatment (Second course) : Tryparsamide, five injections in 3-gramme doses from 7.11.25 to 18.12.25.

Total dosage 15 grammes.

Result : Improvement. Then relapse in April, 1926.

Treatment (Third course) : Tryparsamide, eight injections in 2 to 3-gramme doses.

Total dosage 20 grammes.

Result : Died August, 1926.

The results obtained in the three classes are summarised in the following tables :—

TABLE I.

Showing cases which received a single injection of Bayer 205 only.

Category	Total treated	Dead	Well
Class 1	1	1	0
Class 2	1	0	1
Class 3	3	3	0

TABLE II.

Showing cases which received three injections of Bayer 205 over a period of 15 to 30 days and a total amount of at least 3 grammes (children receiving proportionately smaller doses).

Category	Total treated	Dead	Alive	
			Relapsed	Well
Class 1	8	3	0	5
Class 2	7	6	1	0
Class 3	2	2	0	0

NOTE.—Cases who were subsequently treated with Tryparsamide for relapse are also included.

TABLE III.

Showing cases which received at least eight injections of Tryparsamide over a period of eight weeks and a total amount of at least 24 grammes.

Category	Total treated	Dead	Alive	
			Relapsed	Well
Class 1	1	0	0	1
Class 2	2	1*	1	0
Class 3	0	0	0	0

* Died of pneumonia.

TABLE IV.

Showing cases which received at least three injections of Bayer 205 over a period of 15 to 30 days and of a total amount of at least 3 grammes, followed within two months by a course of at least ten injections of Tryparsamide and a total amount of at least 29 grammes (children receiving proportionately smaller doses).

Category							Total treated	Dead	Well
Class 1	1	0	1
Class 2	4	3	1
Class 3	1	0	1

TABLE V.

Showing cases which received an incomplete course of Bayer 205 and Tryparsamide.

Category							Total treated	Dead	Alive	
									Relapsed	Well
Class 1	13	2	2	9
Class 2	4	1	1	2
Class 3	2	0	0	2

TABLE VI.

Showing relapsed or re-infected cases after further treatment.

Treatment after relapse	Died	Recovered
At least 3 grammes of Bayer 205 or Fournau 309 in three or more injections	5*	0
Irregular course of Tryparsamide or Bayer and Tryparsamide	4	4

* Two of these cases, belonging to Class 1, are not included in the preceding tables as they received their first course of Bayer irregularly.

RESULTS OF TREATMENT

Before discussing the results obtained it is necessary to make clear the position with regard to relapses. In none of the relapsed cases could re-infection be excluded, and not until a large series of treated cases is observed under fly-free conditions can data be obtained which may distinguish the two conditions.

It may, nevertheless, be of significance that it is the late cases that usually 'relapse.' We may, perhaps, assume that re-infections, if they have occurred, bear the same ratio to true relapses throughout the three classes.

In general, the tables show that while early cases, Class 1, treated with Bayer 205 alone, have, in the majority of instances (over 60 per cent.) recovered, late cases (Classes 2 and 3) so treated, almost invariably died, whereas equally late cases treated with both Bayer and Tryparsamide have occasionally recovered.

The more advanced the cases before treatment, the greater the tendency to relapse and death.

A relapse, when it occurs, usually becomes evident within less than a year from the commencement of treatment, but may be as late as fifteen months or more. There is no evidence yet that relapses occur after two and a half years, but that they may do so in some form or other is possible in view of the fact that, in cases of apparent recovery, lumbar puncture performed nearly two years after the commencement of treatment sometimes reveals a large number of cells (as many as 260 per c.mm.) in the cerebro-spinal fluid.

One point in connexion with relapses may be of practical importance: namely, that parasites sometimes appear in the peripheral blood when the patient feels in almost normal health and is going about his ordinary duties. If these parasites are infective these patients may be a greater danger to the community than if they had been left untreated.

TOXIC EFFECTS OF THE DRUGS

Beyond the severe pyretic reaction that frequently follows the first injection of Bayer 205, the only toxic symptoms seen after this drug were Nephritis and a combination of Dermatitis and Stomatitis.

It was not possible to make regular examinations of the urine and some transitory albuminurias may have been overlooked. Severe long-standing Nephritis was seen in only three cases, one of whom had Dermatitis. In two of these the Nephritis appeared to be a contributory cause of death. Only one case of Dermatitis was seen and it has already been described in a previous paper.

The only toxic effect of Tryparsamide seen was Optic Neuritis. This was nearly always bilateral and usually complete and permanent, though perhaps sometimes it may be partial and transitory. This condition may possibly be due, not to the pure drug itself, but to some product of its decomposition, for, though it occurs occasionally when the solution is made in distilled water, it appears to be more common if only filtered water is used, or if the solution is heated to too high a temperature.

CONCLUSIONS

Generally speaking, the earlier the treatment the better the chances of recovery. There is good reason to expect that an uncomplicated case taken in the first two or three weeks of infection will make a complete recovery, if given four grammes of Bayer 205 in three or four doses, the treatment being spread over a month. To allow a margin of safety it may be advisable to administer as much as eight grammes, but if this is done the urine should be watched daily for albumen. How far Bayer 205 should be withheld when albuminuria occurs is a matter for judgment in each individual case, but generally the treatment should cease until the albumen disappears if three grammes have already been given, but should not be withheld for more than ten or fourteen days if only two grammes or less have been given. An individual dose should not ordinarily exceed one and a half grammes. The optimum total dosage of Bayer 205 is not known, and a series of late cases on a prolonged course of treatment should be worth observing.

Tryparsamide alone, though not generally regarded as satisfactory in this type of Sleeping Sickness, has given good results in some cases and would seem to deserve further trial. Solutions of this drug should always be made in distilled or freshly-collected rain

water, and the solution should not be allowed to stand long or heated beyond blood heat. Should dimness of vision occur, treatment should cease until the sight is completely restored.

Treatment by Bayer 205 followed by Tryparsamide gives better results than Bayer 205 alone. When the combined treatment is being given Bayer 205 should be administered in the same way as when the drug is given alone and after this course Tryparsamide should be given in 2 or 3-gramme doses at weekly intervals (with or without a month's interval between the fourth and fifth, and the eighth and ninth injections) until at least 36 grammes are given. What interval should elapse between the last dose of Bayer 205 and the first dose of Tryparsamide is still a matter of conjecture, but a month has been found suitable.

In the above series children generally reacted badly to treatment, but this may possibly be because the doses were too small. It has since been found that children tolerate both drugs extremely well.

It is important that a series of treated cases who are in good health but whose cerebro-spinal fluid remains abnormal for several months after completing treatment, should be given a further course of Tryparsamide without waiting for the development of any symptoms. These could then be compared in years to come with cases that had not been so treated.

Work on the infectivity of Trypanosomes in relapsed cases is urgently needed.

I am indebted to Drs. Buchanan, Park Noble, and Williamson, and to Mr. Irvine, for completing the treatment in some of the cases and for keeping them under observation; to Dr. Coghlin, for reporting on the Ufipa cases, in 1928.

I have to thank Dr. J. O. Shircore, C.M.G., Director of Medical Services, Tanganyika Territory, for permission to publish the paper.

REFERENCE

- MACLEAN, G. (1928). A dermatitis associated with 'Bayer 205' treatment of Rhodesian Trypanosomiasis. *Ann. Trop. Med. & Parasitol.*, **22**, 531.

THE ACTION OF PRÄP. 3510 IN RHODESIAN SLEEPING SICKNESS

BY

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In 1928, supplies of a drug known under the name of Präp. 3510 were obtained in Tanganyika Territory, from the firm of I. G. Farbenindustrie A. G., Höchst-am-Main, Germany. Präp. 3510 is a white powder easily soluble in cold water. It is an arsenic compound but its formula is not disclosed.

The drug is made up in 1, 2 and 2½ gram ampoules, but there is no other guide to dosage.

The first series consisted of three cases treated in April and May, 1928. Two of these were chosen because they were very advanced cases for whom there was little hope of recovery with any known treatment. The third case was comparatively robust. He was regarded as a good subject because he appeared to have sufficient reserve of vitality to react to Bayer 205 later if the new drug did not prove efficacious. He was also suffering from Keratitis, and this fact seemed an additional advantage in gauging the action of the drug.

The drug was not successful and all three patients eventually died. This was to be expected in the first two, who could hardly hope to recover unless the new drug proved more efficacious than Bayer 205. The death of the third was rather a surprise and was probably accelerated by the action of the drug.

The following is a brief history of each case:—

CASE 1. Male. Age about 30 years. Ill about 2½ months. Condition on the day treatment commenced: Just able to walk. Some emaciation but no oedema. Auxiliary glands slightly enlarged. Fresh blood—Trypanosomes present in fair numbers.

Treatment with Präp. 3510: 3·75 gms. intravenously.

First day after treatment: Vomited during the night. Fresh blood—No trypanosomes in 150 fields. Cerebro-spinal fluid—Trypanosomes present. Cells 25 per c.mm.

Second day after treatment : Vomiting ceased. Fresh blood—No trypanosomes in 150 fields.

Fourth day after treatment : Vomited again through the night. Fresh blood—No trypanosomes in 200 fields.

Sixth day after treatment : Vomiting ceased during the previous day. Fresh blood—No trypanosomes in 200 fields. Became rapidly weaker and died in the afternoon.

CASE 2. Male. Age about 40 years. Ill about 2 months. Condition on the day of treatment : Just able to walk. Emaciated. Slight oedema of feet. Epitrochlear glands enlarged. Fresh blood—Trypanosomes in large numbers.

Treatment with Präp. 3510 : 2.5 gms. intramuscularly.

First day after treatment : Vomited through the night. Fresh blood—No trypanosomes in 200 fields. Cerebro-spinal fluid—Trypanosomes present. Cells 45 per c.mm.

Third day after treatment : Vomited yesterday. Weaker. Fresh blood—No trypanosomes in 200 fields.

Fifth day after treatment : Vomiting ceased. Fresh blood—No trypanosomes in 200 fields.

Sixth day after treatment : Stuporose.

Seventh day after treatment : Comatose. Fresh blood—No trypanosomes in 200 fields.

Eighth day after treatment : Died.

CASE 3. Male. Age about 20 years. Ill about 2½ months. Condition on the day of treatment : Well nourished. Able to walk for miles. No oedema. Spleen, axillary and epitrochlear glands enlarged. Conjunctivitis and keratitis of one eye. No vomiting. Fresh blood—Trypanosomes scanty.

Treatment with Präp. 3510 : 2.25 gms. intramuscularly.

First day after treatment : Vomited the previous evening after the injection. Fresh blood—No trypanosomes in 160 fields. Cerebro-spinal fluid—Trypanosomes present. Cells 150 per c.mm.

Third day after injection : No vomiting for 48 hours. Eye condition distinctly improved. Fresh blood—No trypanosomes in 200 fields.

Sixth day after treatment : No more vomiting. Conjunctivitis and keratitis almost completely disappeared. Patient feels well. Fresh blood—Trypanosomes numerous.

Seventh day after treatment : Fresh blood—Trypanosomes still numerous but are rather sluggish. After examination of the blood, 2 gms. of Präp. 3510 was given intramuscularly. Vomiting set in shortly after administration but was checked with Sodium Bicarbonate and Opium.

Eighth day after commencement of treatment : No vomiting, but weaker than before second injection. Stained thick blood film—No trypanosomes.

Ninth day after commencement of treatment : Weaker. Fresh blood—No trypanosomes in 180 fields.

Tenth day after commencement of treatment : Died.

The reactions observed in Case 3 suggested that the symptoms following the administration of the drug might not be due to direct action, but to the products of the sudden destruction of large numbers of trypanosomes.

As the drug appeared to have a definite trypanosomicidal action, it was decided that observations ought to be continued under modified conditions. A fourth case was accordingly selected and 1 gm. of Bayer 205 was administered to sterilise the peripheral blood.

A week after, 2 gms. of Präp. 3510 was administered intravenously. In this case there was little or no reaction, and the patient was able to take eight weekly intravenous injections, a total of 16 gms., without untoward symptoms and with very good clinical results. Nine months after commencing these injections he felt well except for some pains in his legs, and he was doing heavy muscular work.

Two more cases were subsequently treated with Präp. 3510 alone : at first, 1 gm. doses were given, but later the doses were increased to 2 gms. These cases survived the treatment, but the results were not satisfactory as the following histories show :—

CASE 5. Male. Age about 60 years. Ill about 1 month. Condition on the first day of treatment : Fairly well nourished. Able to walk for miles. Oedema legs and feet. Axillary and epitrochlear glands enlarged. Fresh blood—Trypanosomes present in fair numbers. Cerebro-spinal fluid—Trypanosomes present in fair numbers. Cells 30 per c.mm.

Treatment with Präp. 3510 : 1 gm. intravenously.

First day after treatment : No vomiting. Only symptom was a rise of temperature about five hours after injection. Fresh blood—No trypanosomes in 150 fields. Stained thick film also negative.

Subsequent history of the case : The blood was again negative on the third and twelfth days after treatment, and no untoward symptoms developed.

On the fourteenth day after commencement of treatment a second 1 gm. dose of Präp. 3510 was given. 2 gms. were given on the twenty-first day, 2 on the twenty-eighth, and 1½ on the thirty-fifth.

Trypanosomes were present in the blood after the second and third injections and in the cerebro-spinal fluid after the fifth.

No vomiting occurred even after the larger doses but the general condition of the patient had not improved, and routine treatment with Bayer 205 and Tryparsamide was resorted to.

CASE 6. Male. Age about 25 years. Ill about 3 months. Condition on the first day of treatment : Nutrition and general health good. Trace of oedema of legs and feet. Able to do a moderate amount of work. Blood (fresh and

stained)—no parasites seen. Cerebro-spinal fluid—Trypanosomes (70 per c.mm.) present. Cells 5 per c.mm.

Treatment with Präp. 3510 : 1 gm. intravenously.

First day after treatment : No vomiting. Stained thick blood film—No trypanosomes.

Subsequent history of the case : Trypanosomes were found in the blood twelve days after treatment, but no untoward symptoms developed.

On the fourteenth day after commencement of treatment a second 1 gm. dose of Präp. 3510 was given. 2 gms. were given on the twenty-first day, 2 on the twenty-eighth, and 1½ on the thirty-fifth.

Trypanosomes were present in the blood after the second and third injections, and in the cerebro-spinal fluid after the fifth.

During the first month of treatment there was a slight improvement in the general health, but after the fifth injection, patient began to lose ground rapidly, and it became necessary to revert to Bayer 205 treatment.

There was no vomiting after the larger doses of Präp. 3510.

CONCLUSIONS

The limits of the pharmacological dose of Präp. 3510 in man are not known, but there is reason to suspect that its action is much more toxic if there are large numbers of trypanosomes in the peripheral circulation when it is administered.

It has proved much less efficacious than either Bayer 205 or Tryparsamide in moderate doses. While it may possibly be an efficient trypanosomicide in larger doses the margin of safety is so narrow that it is of no practical value when administered alone. Its action in association with Bayer 205 is being observed, but it will probably take some years before any definite conclusions can be arrived at about this method of treatment.

The drug is well worth a trial in the various trypanosomiases of domestic stock.

I am indebted to Dr. Fairbairn for keeping Cases 5 and 6 under supervision during the latter part of their treatment.

I have to thank Dr. J. O. Shircore, C.M.G., Director of Medical Services, Tanganyika Territory, for permission to publish the paper.

AN ACCOUNT OF THE ANATOMY OF CERTAIN CESTODES BELONGING TO THE GENERA *STILESIA* AND *AVITELLINA*

BY

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PLATE V

INTRODUCTION

In the museum of the Liverpool School of Tropical Medicine there is a large collection of cestodes referable to the sub-family AVITELLININAE. The bulk of the material was collected from Africa.

Investigations on these worms were suggested by Dr. T. Southwell, F.R.S.E., of the Liverpool School of Tropical Medicine, to whom I am also indebted for much help and criticism. I am also indebted to Professor W. Yorke, M.D., for much valuable advice and help.

The material was preserved in formalin solution and was all fragmented, no complete worms being present and, owing to this fact, it proved a laborious task to follow the development and relationships of the different organs in the species described.

A large number of total mounts, serial, transverse, horizontal and sagittal sections of the different parts of the worms were made. The thickness of the sections varied according to circumstances, from 5μ to 25μ . The stain used throughout the work was acetic acid alum-carmine.

As many camera lucida drawings as possible were made because, in my opinion, this is the surest and easiest means of identifying these worms. Furthermore, the preparation of these figures assisted materially in the elucidation of several points which were not at first perfectly clear.

Family ANOPLOCEPHALIDAE Fuhrmann, 1907.

Scolex unarmed, without rostellum or accessory suckers. Segments broader than long. Genital organs single or double in each segment. Genital pores may be absent. Genital ducts generally pass dorsally to the excretory vessels, but may pass between them or ventrally to them. Testes numerous or few. Uterus tubular, reticulate or sac-like; it may become transformed into egg-capsules, or it may be replaced by one or more paruterine organs. Eggs with three envelopes, the inner one being chitinous and sometimes bearing a pyriform apparatus. Adults in birds, mammals and reptiles. In no species of this family is the life-history known.

Type genus :—*Anoplocephala* Blanchard, 1841.

KEY TO SUB-FAMILIES (after Southwell).

Uterus persistent	<i>Anoplocephalinae</i>
Uterus developing paruterine organs	<i>Thysanosominae</i>
Uterus breaks up into egg capsules	<i>Linstowinae</i>

Sub-family THYSANOSOMINAE Fuhrmann, 1907.

Large worms. Genital pores double or single; in the latter case they are irregularly alternate. Genital canals dorsal to excretory vessels or between them. Testes very numerous or few, in a single field, or in two lateral groups. Female genitalia in poral half of segment. Vitelline gland may be absent, in which case the ovary contains the nutritive cells. Uterus tubular, sometimes very long, and undulating. Paruterine organs present; may be very numerous or single. They each contain several eggs. Adults in ruminants.

Type genus :—*Thysanosoma* Diesing, 1835.

KEY TO THE GENERA INCLUDED IN THE SUB-FAMILY THYSANOSOMINAE (after Southwell).

With a double set of genital organs in each segment.....	<i>Thysanosoma</i>
With a single set of genital organs in each segment	1
1. With one paruterine organ in each segment	<i>Avitellina</i>
With more than one paruterine organ in each segment.....	2
2. With two paruterine organs in each segment	<i>Stilesia</i>
With numerous paruterine organs in each segment	3
3. Testes within excretory vessel	<i>Ascotaenia</i>
Testes in two fields, one lateral, to the excretory-vessels on each side	<i>Helictometra</i>

Baer (1927) united the sub-family AVITELLININAE Gough, 1911, with the sub-family THYSANOSOMINAE Fuhrmann, 1907.

The following genera referable to this old sub-family AVITELLININAE have been described, viz., *Stilesia* Railliet, 1893, *Avitellina* Gough, 1911, *Hexastichorchis* Blei, 1921, and *Anootypus* Woodland, 1928.

Southwell (1929) considered that only two genera are valid, viz., *Stilesia* and *Avitellina*, and he placed the genera *Hexastichorchis* and *Anootypus* as synonyms of the genus *Avitellina*. This paper deals with species of the two genera *Stilesia* and *Avitellina*.

Diagnosis of the genus *Stilesia* Railliet, 1893.

Strobila thin and narrow. Outer segmentation always distinct, and corresponding to the internal segmentation of the genitalia. Longitudinal muscles in one layer. Ventral excretory vessels lateral to dorsal vessels. Testes in two groups, one on each side of the strobila, all external to the dorsal excretory vessels, none in the middle field. Cirrus sacs ventral and anterior to the vulvae. Genital ducts pass between the excretory vessels and dorsal to the nerve. Ovary single, situated porally, internally to the ventral and externally to the dorsal excretory vessels. Uterus single but with two lateral dilatations, each situated between the dorsal and the ventral excretory vessels. Paruterine organs double, and taking the place of the lateral dilatations of the uteri. Parasites in ruminants.

Type species :—*Stilesia globipunctata* (Rivolta, 1874), Railliet, 1893.

KEY TO THE KNOWN SPECIES OF THE GENUS *Stilesia*.

Testes all lateral to the dorsal excretory vessels	<i>S. hepatica</i>
Testes all lateral to the ventral excretory vessels	I
Vas deferens forms a mass of intricate convolutions between the cirrus pouch and the outer wall of the ventral excretory vessels...	<i>S. vittata</i>
Vas deferens forms three or four close coils before it enters the cirrus pouch	<i>S. globipunctata</i>

Diagnosis of the genus *Avitellina* Gough, 1911.

Strobila thin and narrow. Outer segmentation usually not distinct. Longitudinal muscles in a single layer in the cortex and well developed ; another thin subcuticular layer may or may not be

present. Testes in two or four rows. Uterus and paruterine organs single in each segment. Genital ducts pass dorsal to the excretory vessels and nerve.

Type species :—*A. centripunctata* (Rivolta), 1874, Railliet, 1893.

KEY TO THE SPECIES OF THE GENUS *Avitellina*.

Dorsal excretory vessels completely absent	1	
Dorsal excretory vessels present	2	
1. With four rows of testes		<i>A. edisfontaina</i> Woodland, 1928
With two rows of testes		<i>A. ricardin</i> Woodland, 1928
2. Dorsal excretory vessels lateral to ventral excretory vessels ...	3	
Dorsal excretory vessels median to ventral excretory vessels ...	4	
3. Some testes lateral to the dorsal excretory vessels		<i>A. pintneri</i> Blei, 1921
No testes lateral to the dorsal excretory vessels		<i>A. aegyptiaca</i> n.sp.
4. Outer row of testes one testis deep	5	
Outer row of testes more than one testis deep	6	
5. Mature paruterine organs kidney-shaped		<i>A. sudanea</i>
Immature paruterine organs snail-shaped		<i>A. laborea</i> Woodland, 1927
6. Outer rows of testes are four to eight deep, and there is a distinct row of testes external to the nerve cords.....		<i>A. southwelli</i> n.sp.
Outer rows of testes three to six deep ; paruterine organs are like bunches of bananas		<i>A. goughi</i> Woodland, 1927
Outer rows of testes are two to three deep and with anterior annular thickenings on each proglottid		<i>A. chalmersi</i> Woodland, 1927
Outer rows of testes one to two deep ; paruterine organs sac-like		<i>A. centripunctata</i>

(1) *Stilesia globipunctata* (Rivolta), 1874, Railliet, 1893.

Host :—*Ovis laticauda*.

Locality :—Unknown, probably Africa.

The worms are whitish in colour and delicate. The margins are serrated on account of the fact that the posterior angles of the segments project laterally, thus distinguishing the anterior from the posterior ends of the pieces of the strobila with certainty. The breadth of the immature parts of the strobila is about 330μ . The individual segments are about 80μ to 90μ in length. The male mature strobila measure about 864μ in breadth and the individual segments 144μ to 196μ in length. In the region where the paruterine organs are well developed, the breadth of the worm varies from 490μ to 900μ and the segments are about 220μ in length in the narrow parts, and about 110μ in length in the broader parts. The genital pores are irregularly alternate and open near the anterior angles of each segment.

Excretory System. Both excretory vessels are well developed throughout the whole length of the strobila. The ventral vessels are lateral to the dorsal vessels and measure, in the immature region, about 10μ in diameter. The dorsal vessels have thicker walls than the ventral ones and are smaller, measuring only 7μ in diameter ; they lie slightly dorsal to the ventral vessels.

In the male mature region, however, the ventral vessels enlarge to about 30μ and the dorsal vessels to about 10μ in diameter. In the region where the paruterine organs are developed, the ventral vessels measure about 40μ and the dorsal vessels only 6μ in diameter, but the latter are quite distinct.

Muscular System. The longitudinal muscles are in one layer and are feebly developed except in the anterior part of the worm, where they are well developed.

Male Genitalia. The testes are situated lateral to the ventral excretory vessels. They are more or less restricted to the angles formed by the ventral excretory vessels and the posterior borders of the segments. There are from four to seven on each side.

Porally they are always posterior to both the cirrus pouch and the vulva, and they never occur anterior to these organs. The testes on the aporal side usually out-number those on the poral side. Each fully mature testis measures about 47μ in diameter.

The cirrus pouch is pyriform and measures 54μ by 43μ ; it is always anterior and ventral to the vulva.

The vas deferens is thrown into three or four coils before it enters the cirrus pouch.

Female Genitalia. The ovary is situated between the dorsal and ventral excretory vessels on the poral side, and at first is indicated by a cluster of darkly-staining nuclei. It measures about 58μ by 43μ when fully mature.

Immediately posterior to it is another cluster of darkly-staining nuclei indicating the rudiments of the poral part of the uterus, and in the corresponding position in the aporal half of the segment a similar uterine rudiment is present. Each of these measures about 25μ in diameter. Later on the uterus becomes a spherical or oval mass of fibres measuring about 35μ in diameter and yet containing no eggs.

In the segments immediately posterior a very interesting

phenomenon is displayed, in that the two uteri enlarge at the expense of the ovary. That is to say, in the succeeding twenty to fifty segments the ovary gradually atrophies, the two uteri enlarge and finally contain the eggs, the ovary having completely disappeared. The eggs can be seen passing from the ovary to the poral part of the uterus, which latter lies posteriorly and in close contact with it. No connecting duct was seen between them: the eggs most probably pass through the tissues. In several segments eggs were seen wandering towards the *aporal* parts of the uteri and a few of them were distinctly amoeboid in shape. In some cases the duct connecting the two parts of the uterus was seen containing the wandering ova; in other cases, however, it could not be traced although eggs were seen in the middle of the segments. This duct is tortuous in its course, appears to be contractile and measures about 5μ only in the region where there are no ova. The writer must state here that the figures 8 and 10 of *Stilesia vittata* and *Stilesia globipunctata* in Gough's monograph are erroneous with regard to the position and relation of the uteri to the ovaries. He figures the uteri anterior and median to the ovaries, but they are without doubt immediately posterior to them, as shown in fig. 2.

Paruterine Organs. In partly-gravid strobila the paruterine organs are situated between the dorsal and ventral excretory vessels and are attached posteriorly to fibrous structures, which latter assume two shapes, one being like the letter T and the other resembling an inverted letter L. The short limb of the L-shaped fibrous structure is directed inwardly. If we trace these structures posteriorly, we find that the cross-bar of the T-shaped rudiment or the short limb of the L gradually expands and separates from the remaining limb of the T or the long limb of the L, forming a strong fibrous pad in front of the paruterine pouch to be described. The remaining limb of the T or the long limb of the L develops, and a membrane round the fibres is differentiated, forming in the end the paruterine pouch. This is situated anteriorly and somewhat median to the paruterine organ and is composed of concentrically arranged fibres. An opening between this pouch and the paruterine organ is present and through it some of the fibrous tissue passes into the paruterine organ, pushing the eggs towards its posterior margin. In some paruterine pouches, especially those at the posterior

end, a number of eggs, two or three, can be seen in the middle of the concentric fibres.

The paruterine organ also changes its position from being median to the ventral excretory vessel to being dorsal, or even completely external to it in some segments; being pushed, so to speak, by the developing paruterine pouch, which, so far as can be seen, is located between the dorsal and the ventral excretory vessels.

Stiles, in his description of *Stilesia globipunctata*, says:— 'An organ, the function of which I am unable to explain, appears anterior to the uteri and running transversely. This organ lies ventrally of the vas deferens—in those cases where the vas deferens extends so near the anterior margin and stains quite dark in carmine or haematoxylin.' I am of opinion that these organs are the fibrous pads referred to in this paper.

(2) *Stilesia vittata* Railliet, 1896.

Host:—*Camelus* sp. (Camel).

Locality:—Unknown.

The strobila to the naked eye appears quite different from that of *Stilesia hepatica*. It is more transparent and less fleshy. Segmentation is visible to the naked eye.

The fragments examined measure up to 10 cm. in length and from 2.5 mm. to 4 mm. in breadth. All the segments are broader than long, and measure about 215μ in length.

The fragments are from different parts of the strobila and nearly all of them are folded ventrally on themselves and much curled and twisted.

Strong fibrous pads are situated towards the anterior edges of gravid segments; each pad is composed of a middle part and two denser, saucer-shaped extremities, the concavities of which are directed posteriorly, covering the inner parts of the paruterine organs. The genital pores are irregularly alternate and open near the anterior angles of the segments.

Excretory System. The ventral excretory vessels are well developed, especially in the posterior parts of the strobila, where they measure about 214μ in diameter, whilst anteriorly they measure about 73μ .

The dorsal vessels, on the other hand, are only well developed

in the anterior part of the worm, and measure about 23μ in diameter and have thick walls. They are situated internally to the ventral excretory vessels, and either slightly dorsal to them or at the same level.

Posteriorly the dorsal vessels gradually atrophy and the ventral enlarge.

In each segment there arise from the outer and inner sides of both ventral vessels, two small, thin-walled tubes, having a diameter of about 7μ . Each arises either singly or as the result of the fusion of two small ducts. The internal branch joins the main ventral vessel on the other side, being situated towards the posterior margin of the segment. The outer branch can be traced towards the margin of the segments where it divides into three or four branches. Like the internal branch it is situated near the posterior margin of the segment.

Nervous System. The nerve cord is situated about midway between the lateral margin of the strobila and the outer wall of the ventral excretory vessel.

Muscular System. The longitudinal muscles are in one layer only, not in two as described by Gough. This layer is very weakly developed. A subcuticular layer is not present. The transverse muscles are also feebly developed and separate the denser, cortical parenchyma from the spongy medulla. Strong and distinct muscular fibres run from the wall of the genital atrium to the dorsal and ventral surfaces of the segment, and ramify before reaching the ventral or dorsal surfaces. They are undoubtedly modified dorso-ventral fibres.

Calcareous Corpuscles are irregularly scattered throughout the parenchyma but tend to be clustered towards the junction of the segments, more so towards the margins. In cross-sections they are seen to be situated mostly in the cortical parenchyma. They stain very lightly and most of them show a concentric structure. They are irregular in shape and measure from 7μ to 10μ .

Male Genitalia. The testes are spherical or oval in shape and are situated externally to the ventral excretory vessels. There are from five to ten on each side, each measuring about 66μ in diameter. On account of the fact that the male and female genital ducts occupy the anterior half of the segment on the poral side,

the testes of this side are situated in the posterior half, whilst on the aporal side they extend much more anteriorly. It is noteworthy that the testes have not atrophied even in segments in which the paruterine organs are well developed. The vas deferens, which has not previously been described, is clearly shown in our *toto* mounts. It begins on the aporal side as a thin tube having a diameter of about 7μ . It appears to arise from the innermost testis of the aporal side and runs across the anterior part of the segment, in front of the vessel connecting the two ventral excretory vessels. In the middle field it is very convoluted and stains more deeply than the rest of the duct, thus making this part easily seen even under a low magnification. More laterally towards the pore side it again continues its more or less straight course. It crosses the posterior margin of the poral paruterine organ and then bends towards the anterior margin in front of the testes, forming a mass of intricate convolutions, before it enters the cirrus pouch, i.e., between the pouch and the outer wall of the ventral excretory vessel. As far as can be seen there is no capsule surrounding this mass of convolutions. Immediately internal to this mass of convolutions the vas deferens dilates into a small oval vesicula seminalis with thick walls, the contents of which stain deeply—these are probably spermatozoa.

The vas deferens could only be seen in a few segments; the middle convoluted part is, however, visible in many others. The cirrus pouches are oval in shape and open towards the anterior angles of the segments. They measure about 115μ by 66μ . The cirrus was protruded in some of the segments and measured about 83μ in length.

Female Genitalia. The ovary is only found in segments where the paruterine organs are not yet developed. It is situated on the poral side only, between the ventral and dorsal excretory vessels but nearer the former. It measures about 100μ by 66μ and contains from twenty to thirty ova.

The vulvae measure about 82μ by 13μ and are dorsal and posterior to the cirrus pouches on both sides.

The genital atria are about 66μ in depth and their walls are covered by long, slender, hair-like processes which measure about 33μ in length. The genital pores open in the centre of a conspicuous papilla which is elevated from the surface of the margins.

The uterus consists of two dilated lateral parts, one on each side, which are situated between the dorsal and ventral excretory vessels. There can be no doubt that these two dilatations are connected by a duct similar to that found in *Stilesia hepatica* and *Stilesia globipunctata*, but unfortunately it could not be traced in our specimens, in spite of the fact that many transverse sections were made in different regions of the strobila. Paruterine organs take the place of the uteri in posterior segments: there are two in each segment, one internally to each ventral excretory vessel and in close apposition to its internal wall.

(3) *Stilesia hepatica* Wolffhügel, 1903.

Host :—(1) *Cobus cob*, gall bladder and bile ducts; East shore, Lake Albert, Tonya, Africa; (2) Liver of sheep, Durban, South Africa; (3) Hepatic duct of sheep, Rhodesia.

The breadth of the fragments range from 1.3 mm. to 2.4 mm. Segmentation is quite distinct to the naked eye. The margins of the strobila are serrated on account of the projection of the posterior angles of the segments. The latter are much shorter than broad, measuring from about 40μ in male mature segments, up to 200μ in length in gravid segments. The posterior borders of most of the segments are excavated, forming shallow concavities into which the anterior borders of the succeeding segments lie.

The genital pores are irregularly alternate and situated near the middle of the lateral margin of the segments.

Excretory System. The excretory vessels are both well developed; the ventral ones are situated externally and ventrally to the dorsals and are about 66μ in diameter. The dorsal vessels have thicker walls than the ventral ones and they measure about 40μ in diameter. In no other species of the genus *Stilesia* are the dorsal vessels so well developed and constant, or are the walls so thick.

Nervous System. The nerve cord is situated externally to the ventral excretory vessels and is nearly midway between the margin and the outer border of the ventral vessel.

Muscular System. The longitudinal muscles consist of a stout layer which almost surrounds the strobila. It is situated about 132μ below the cuticle and measures about 99μ in thickness.

Male Genitalia. The testes are oval in shape, measuring about

116 μ by 46 μ in diameter when fully mature. They are arranged in two rows, one on each side of each segment. From eight to ten testes can be counted on each side. They are situated laterally to the dorsal excretory vessels and dorsally to the ventral vessels.

The vas deferens from the aporal testes meets that from the poral testes, forming by their union the main vas deferens. This passes towards the poral side between the two excretory vessels and dorsal to the nerve. It forms a few loops dorsally to the ventral excretory vessel before it enters the cirrus pouch. The latter is pear-shaped, measuring 83 μ in length by 50 μ in maximum diameter.

Female Genitalia. The ovary is spherical or kidney-shaped and measures 80 μ by 50 μ . It is situated porally just median to the ventral vessel and nearly on the same level. It lies laterally and ventrally to the dorsal vessel. The uterus is single but consists of a transverse tube dilated at each extremity; each dilatation is oval, measures 66 μ by 50 μ , and is situated between the dorsal and the ventral excretory vessel on each side. The two parts are connected by a duct through which ova travel from the poral to the aporal parts of the uterus. The paruterine organs are two in number, one on each side, and they measure about 132 μ by 100 μ .

(4) *Avitellina centripunctata* (Rivolta), 1874, Railliet, 1893.

Host :—Ox.

Locality :—Freetown, West Africa.

The fragments examined measure about 2.5 mm. in breadth. The margins appear slightly serrated, but when the fragments are suitably magnified, these indentations are found not to correspond to the segments, as two or three genital openings may occur between two indentations. This is the case in the male mature segments, but in the gravid ones the indentations are not so deep and the margins are only raised here and there, especially where the genital pores open.

With regard to Woodland's statements that 'in gravid proglottids of *A. centripunctata* the margins are more salient posteriorly and are crenulated on their anterior borders,' and (in a footnote), 'curiously enough, Gough's figure of the Transvaal species described by him shows crenulations on the posterior border of the margin,' it is to be noted that our specimens do not show these characteristics of the margins described by Woodland and Gough. The genital

pores are irregularly alternate, and the segments are very short ; in male mature and gravid segments there are no septa formed between the segments, although Woodland figures them. In gravid segments, however, strong fibrous pads, which stain deeply, are formed in front of the paruterine organs.

Excretory System. This is composed of four longitudinal vessels, two on each side. The external pair, the so-called ventral vessels, are large, and can be seen with the naked eye, as two clear lines in stained total mounts. Each measures, in cross-section, about 120μ by 50μ in the male mature region, and 200μ by 150μ in the gravid region, and they have thin walls. The internal pair, the so-called dorsal vessels, are very small in comparison with the ventral ones, and can only be seen under high magnification. In transverse sections they are rendered conspicuous by the presence of clusters of nuclei, which surround them. Attention is here drawn to the position of these so-called dorsal vessels ; they are, in fact, placed ventrally. In order to determine which was the dorsal and which was the ventral surfaces, the writer has always relied on the position of the ovary as being ventrally placed, and on the passage of the genital ducts dorsally to the excretory vessels and nerve. In male mature strobila the dorsal vessel can be seen under high magnification and is situated about 80μ internally to the inner wall of the ventral vessel. In gravid strobila, however, the vessel is very minute, and is situated a distance of about 60μ internally to the inner wall of the ventral vessel, the difference between the two distances being evidently due to the enlargement of the ventral excretory vessels.

Nervous System. This consists of two nerve cords, one external to each ventral excretory vessel, and at one-third the distance from the external wall of the latter vessel and the lateral margin of the strobila.

Muscular System. This consists of an inner thick and an outer thin layer. The former is about 40μ in thickness and is situated about 80μ from the cuticle. It surrounds the strobila except for two gaps at the lateral margins of the worm. The fibres are arranged in pyramid-shaped groups, whose summits are directed outwards. The outer layer is situated under the sub-cuticula, and is one fibre thick.

Male Genitalia. The testes are arranged in four rows, one on each side of each of the ventral excretory vessels. The outer row is one or two testes deep, and does not extend laterally beyond the nerve cord. The inner one is four to five testes deep. The testes are spherical, and have a diameter of about 66μ . The cirrus pouch is pyriform, much shorter than the vagina, and measures about 115μ by 50μ ; it is always anterior to it.

Female Genitalia. The ovaries are spherical in shape, and form two rows in the strobila, one on each side of the medianly-placed uteri. Each ovary measures about 50μ and is situated slightly behind and ventrally to the poral side of the uterus of the same segment.

The vulva is spindle-shaped, and covered by gland cells, which, in surface view, gives it the appearance of a mulberry. It measures about 216μ in length, by 43μ in breadth, and is always situated posteriorly to the cirrus pouch, but it may be either dorsal or ventral to it.

The vagina passes dorsally to both excretory vessels and nerve; it dilates into a receptaculum seminis, midway between the inner wall of the ventral excretory vessel, and the lateral margin of the uterus. It is spindle-shaped, measuring 173μ by 72μ and is often full of spermatozoa. Immediately internal to it, the vagina divides into an oviduct which runs to the posteriorly-situated ovary, and a uterine duct which runs to the uterus; both these ducts have thicker walls than the vagina.

The ovaries and testes atrophy as soon as the paruterine organs develop, the former first.

The uteri and paruterine organs are almost in one row in the middle field of the strobila, but are situated slightly on the poral side. Fibrous pads are yet not developed in front of the paruterine organs in this region. Later on, the uteri become enlarged, sac-like and contain the eggs, but in our specimens no capsules were seen round the latter.

More posteriorly, strong fibrous pads are formed in front of each paruterine organ. These are curved rods of tissue, the concavities of which are directed posteriorly. In the middle of this concavity there lies an oval fibrous organ, the paruterine pouch which measures about 162μ by 108μ .

(5) *Avitellina sudanea* Woodland, 1927.

Host :—*Ovis laticauda*.

Locality :—Unknown ; but the geographical distribution of this host is Tartary, Arabia, Persia, Barbary, Syria and Egypt. These worms were probably collected in Egypt.

The fragments examined range in size from 1 cm. to about 15 cm. in length, by about 2 mm. in breadth. Externally the strobila do not appear to be segmented ; when suitably magnified, the genitalia definitely indicate true segmentation. In the male mature region, the margins of the worm are not indented segmentally, nor are there fibrous septa between the segments ; the latter, however, develop in the gravid part of the strobila.

The genital pores are irregularly alternate.

Excretory System. The ventral vessels appear as comparatively clear, wide canals on each side, and in the immature region they measure about 50μ . The dorsal vessels have a diameter of about 26μ and are situated internally to the former. In a male mature strobila measuring 1.25 mm. in breadth, the ventral vessels are well developed, thin walled, and have a diameter of 80μ . In a gravid strobila they attain a diameter of 250μ , while the dorsals measure only about 4μ in diameter. Thus, antero-posteriorly the ventral vessels enlarge, whilst the dorsal ones become smaller.

Muscular System. The longitudinal muscles are apparently in a single layer. No trace of the outer layer described by Woodland could be found in the specimens examined.

Male Genitalia. The testes are arranged in four rows, one on each side of each of the ventral excretory vessels. The external row is one testis deep, and in the male mature strobila is very much interrupted ; thus in a piece of strobila containing 41 segments, there are, on one side, 19 testes external to the ventral vessels and 84 internal to it. On the other side there are 15 testes external to the ventral vessel and 101 internal to it. It will thus be noted that the outer row of testes is absent in many segments ; in the gravid region, however, it is more continuous. The internal row is two to three testes deep.

Each testis is oval or spherical and measures about 90μ by 60μ in diameter. The vas deferens can be traced to the level of the

ovary and is thrown into a few convolutions. The cirrus pouch is pyriform, about the same length as the vulva, or slightly shorter, and measures 115μ by 36μ . It contains several coils of the vas deferens, which in the gravid segments occupies the entire cirrus pouch. The latter extends to about three-quarters the distance between the lateral margins and the outer wall of the ventral excretory vessel.

Female Genitalia. The ovary is kidney-shaped. In male mature segments it is situated on the poral side of the rudiment of the paruterine organs; it measures about 80μ by 66μ .

The vulva is pyriform and covered, except at its extreme distal extremity, by a layer of glandular cells. These—the minute structure of which can only be seen with an oil immersion lens—are club-shaped, their distal ends being dilated, and each contains a single nucleus.

The vulva measures about 130μ in length, by 40μ in breadth, with the layer of gland cells included, but 19μ only excluding the layer of gland cells.

The vagina is also covered by similar cells, throughout its length, except in the region of the receptaculum seminis; the latter is spindle-shaped, measures about 80μ by 32μ , and is situated immediately median to the ventral excretory vessel. In the gravid strobila it enlarges, and becomes globular, having a diameter of about 100μ ; the glandular cells disappear and it becomes almost filled with spermatozoa. Internally to it, the vagina divides into an oviduct leading to the ovary, and a uterine duct running to the uterus. These two tubes are wider than the vagina.

The vulva is usually posterior to the cirrus pouch in almost all the segments, but is rarely anterior or on the same dorso-ventral plane. Thus, in 150 segments, the vulvae were posterior except in four cases, where they were anterior, and nine cases in the same dorso-ventral plane as the cirrus pouches. The vulvae, again in the majority of the segments, are dorsal to the cirrus pouches; in some they are ventral.

The paruterine organs in this species are of a peculiar shape, as shown in the figures, and anterior to each is an inter-segmental fibrous pad, which stains deeply. Each paruterine organ measures 132μ by 66μ . The eggs are spherical, and measure 23μ in diameter.

(6) *Avitellina southwelli*, n.sp.

Host :—Sheep.

Locality :—Accra, West Africa.

The fragments examined vary in length from 10 cm. to 50 cm., and have a breadth of about 3.2 mm. in the male mature region. The worm is fleshy compared with other species of this genus and, in the gravid region it is almost cylindrical.

In total mounts the lines of segmentation in the male mature region are, at first, very difficult to detect, as there are no septa between them. The genital organs are so crowded together that it was thought in the first instance that each segment contained two ovaries. Close examination by means of horizontal and transverse sections showed, however, that the segments are very short, and contain only one ovary which is situated on the poral side of the uteri.

In gravid strobila, however, the segments are longer, measuring about 72μ in length and indistinct septa are formed between them, which are only visible when horizontal sections are made.

Excretory System. This is composed of the usual pairs of large ventral vessel and the small internally situated dorsal vessels. The former are wide, measuring about 240μ in the male mature region and about 320μ in the gravid region. They follow a very tortuous course. Entire groups of encapsuled eggs, as well as isolated ones liberated from their capsules, are seen inside the ventral vessels in the gravid segments. The dorsal vessels measure about 7μ in the male mature region. They are completely atrophied in the gravid strobila.

Muscular System. The longitudinal muscles are in one layer which is well developed and consists of separate bundles of about twenty fibres each.

Nervous System. This consists of two longitudinal nerves situated one on either side, about midway between the lateral margins of the worm and the outer walls of the ventral excretory vessels.

Male Genitalia. The testes are numerous, compared with other species of this genus; they are spherical and measure from 50μ to 80μ , being arranged in four rows, one on each side of the ventral excretory vessels. The outer row is four to eight testes deep and

there is a distinct row one testis deep external to the nerve cord. This condition, i.e., the presence of testes externally to the nerve cord and of the large number of testes externally to the ventral excretory vessels, has not been described before in any species of the genus *Avitellina*, and, together with other peculiarities to be discussed later, justifies the erection of a new species. The internal row is four to six testes deep and extends from the internal wall of the ventral excretory vessel to the region of the ovaries.

The vas deferens is loosely coiled before it enters the cirrus pouch and runs dorsally to both the excretory vessels and the nerve. The cirrus pouch is pear-shaped and contains several coils of the vas deferens; it measures about 144μ by 36μ .

Female Genitalia. The ovaries are in two longitudinal rows immediately median to the internal row of testes, and in each row they are situated close to each other. Between these two rows are the uteri. As the latter develop the essential genital organs atrophy, the ovaries first.

Each ovary is situated ventrally and internally to the dorsal excretory vessel close to the poral end of the uterus. It is distinctly oval, measuring 70μ by 50μ . Leading from the ovary is a short oviduct which unites with the uterine duct and forms the ootype or fertilization canal. Externally to the latter the vagina dilates to form a receptaculum seminis which is situated dorsally, midway between the dorsal excretory vessel and the poral extremity of the uterus; it is spindle-shaped and measures about 65μ by 36μ .

The vulva is elongated and surrounded by glandular cells; it measures 200μ by 11μ , tapering towards both ends and passing gradually into the vagina.

The genital ducts pass dorsally to both the excretory vessels and the nerve.

The vulva is situated either dorsally or ventrally to the cirrus pouch, and they are on the same plane dorso-ventrally. The uteri lie in the middle fifth in the male mature strobila and the middle third in gravid strobila. At first they are narrow transverse bands of tissue which alternate markedly from side to side. Posteriorly they become sac-like and disposed in two longitudinal rows. Eventually they enlarge laterally and become pear-shaped. When fully gravid they occupy the whole region between the inner walls

of the ventral excretory vessels. Mature paruterine organs measure about 326μ in length by 212μ in maximum breadth, and each contain from three to four egg capsules. The egg measures about 22μ in diameter. This worm differs from all hitherto described species of the genus *Avitellina* in the following points :—

- (1) The outer row of testes is from four to eight deep and there is a row, one testis deep, situated externally to the nerve cord.
- (2) The cirrus pouch and the vulva are in one plane dorso-ventrally.
- (3) It differs from *A. centripunctata* with regard to the position of the uteri in that in *A. southwelli* they alternate from side to side in the male mature as well as in the gravid segments, whilst in *A. centripunctata* they are practically in one row in the male mature region as well as in the gravid strobila.

I propose for this new species the name of *Avitellina southwelli*, in honour of my teacher, Dr. T. Southwell, and in recognition of his unfailing help.

(7) *Avitellina ægyptiaca*, n.sp.

Host :—*Cephalopus* sp. (Duiker).

Locality :—Nagoya, N.E. Rhodesia, Africa.

The fragments examined measure from 2 cm. to 10 cm. in length, and from 1 mm. to 1.8 mm. in breadth. They show no signs of external segmentation, even if suitably magnified, but the genitalia are segmentally arranged. The segments are very short and the genital pores are irregularly alternate.

Excretory System. In an immature portion of strobila, 10 cm. in length and 1.7 mm. in breadth, the arrangement of the excretory vessels is peculiar in that the small dorsal vessels are situated laterally to the large ventral vessels, and in this particular the worm resembles *Hexastichorchis pintneri* Blei, 1921. The dorsal vessel is not coiled; it lies a little nearer to the ventral vessel than to the margin of the worm, and measures, in this region, about 36μ in diameter. The ventral vessels are regularly coiled and measure about 72μ in diameter.

In a fragment of the male mature strobila measuring 5 cm. in

length and 1.6 mm. in breadth, the ventral vessels have a diameter of about 130μ . In transverse sections the two vessels are situated in the same plane, and in the middle line of the dorso-ventral diameter of the strobila.

Muscular System. The longitudinal muscles are arranged in two layers, the inner is strongly developed, and has a thickness of 22μ . The fibres are more or less evenly scattered in this layer, and are not formed into separate bundles. The outer is a subcuticular layer and is situated immediately below the cuticle, and is one fibre deep.

Male Genitalia. The testes are arranged in four rows, one internal and the other external to the ventral excretory vessel, and between the latter and the outer dorsal vessel on each side. The outer row is two to three testes deep, the inner being four or five, and very compact. Each testis is distinctly elongated and measures about 90μ by 54μ .

The vas deferens is thrown into several loose coils before it enters the cirrus pouch. The latter is pyriform and measures about 83μ by 24μ ; it is always dorsal to the vulva on both sides, and on the same plane dorso-ventrally. The genital ducts pass dorsally to both the excretory vessels and the nerve.

Female Genitalia. The ovaries are situated close to the poral side of the uteri, and are ventrally placed; each measures about 54μ . The ovarian eggs measure about 16μ in diameter. The vagina is dilated to form a receptaculum seminis midway between the ventral excretory vessel and the ovary. It is oval in shape and measures about 90μ by 24μ . The vulva measures about 108μ by 11μ , and is not covered by glandular cells.

The paruterine organs, when fully developed, are practically arranged in alternating rows, and occupy the middle third of the breadth of the strobila. Each measures about 245μ by 100μ , and has a semi-lunar fibrous pad in front of it. In gravid segments the testes and ovaries have atrophied and the ventral excretory vessels are straight and not coiled.

This species differs from all the others within the genus *Avitellina* in that the small dorsal excretory vessel lies externally to the ventral vessel although they are in the same transverse plane, as seen in the cross-sections. It agrees with Blei's species, *Hexastichorchis pintneri*, in this respect. It differs from it, however, in having only four rows

of testes, one on each side of each ventral excretory vessel, the outer being situated between it and the dorsal vessel ; thus, there are no testes externally to the dorsal vessel as there are in *H. pintneri*. In the latter species there are six rows of testes in the anterior part of the worm, one internally to the ventral vessel ; one between it and the dorsal vessel, and one external to the dorsal vessel. It differs from *A. centripunctata*, as above stated, in the presence of the small dorsal vessel, lateral to the large ventral vessel ; it also differs from it in the presence of the paruterine organs in two rows in the middle field of the strobila and in the cirrus pouches being always dorsal to the vulvae and in one plane with them dorso-ventrally.

EXPLANATION OF LETTERING

<i>c.</i>	= cirrus.	<i>o.l.m.</i>	= outer longitudinal muscle.
<i>c.c.</i>	= calcareous corpuscles.	<i>p.o.</i>	= paruterine organ.
<i>c.p.</i>	= cirrus pouch.	<i>p.p.</i>	= paruterine pouch.
<i>d.e.v.</i>	= dorsal excretory vessel.	<i>r.s.</i>	= receptaculum seminis.
<i>e.</i>	= eggs.	<i>t.</i>	= testes.
<i>f.p.</i>	= fibrous pad.	<i>t.m.</i>	= transverse muscle.
<i>g.a.</i>	= genital atrium.	<i>ut.</i>	= uterus.
<i>i.f.p.</i>	= intersegmental fibrous pad.	<i>ut.d.</i>	= uterine duct.
<i>i.l.m.</i>	= inner longitudinal muscle.	<i>v.</i>	= vagina.
<i>int.d.</i>	= interuterine duct.	<i>v.d.</i>	= vas deferens.
<i>l.m.</i>	= longitudinal muscle layer.	<i>v.e.v.</i>	= ventral excretory vessel.
<i>n.</i>	= nerve.	<i>v.s.</i>	= vesicula seminalis.
<i>o.</i>	= ovary.	<i>vu.</i>	= vulva.
<i>od.</i>	= oviduct.		

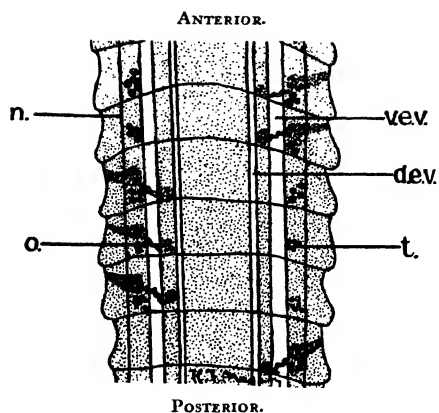


FIG. 1. *Stilesia globipunctata*. Immature segments showing rudiments of the genitalia. $\times 100$.

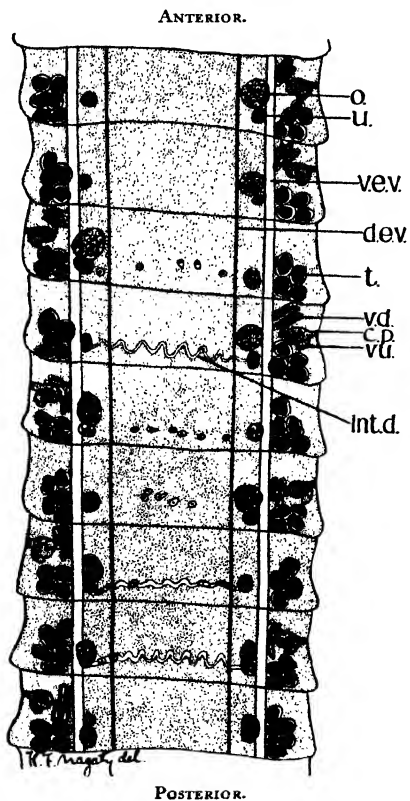


FIG. 2. *Stilesia globipunctata*. Male mature segments showing migrating ova. $\times 66$.

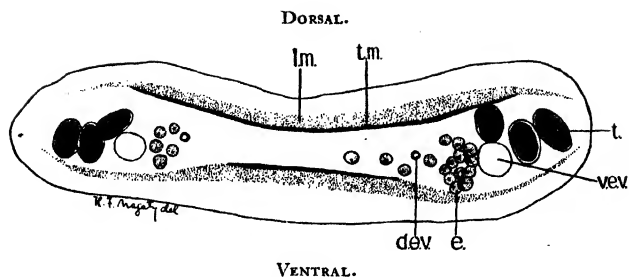


FIG. 3. *Stilesia globipunctata*. Cross-section showing migration ova. $\times 45$.

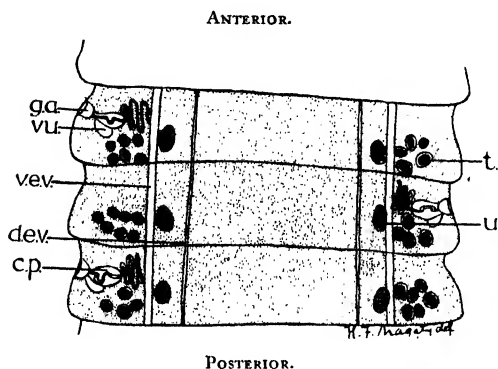


FIG. 4. *Stilesia globipunctata*. Showing fully-developed uteri and the relation of the vulva to the cirrus pouch. The ovaries have completely atrophied. $\times 33$.

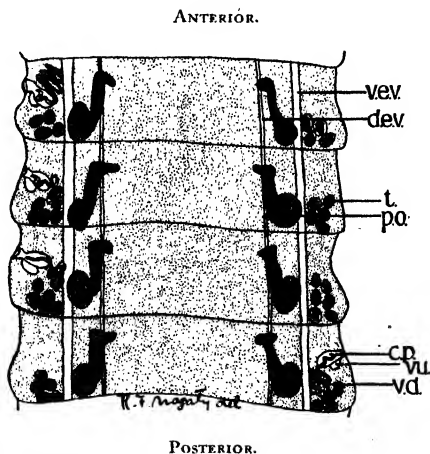
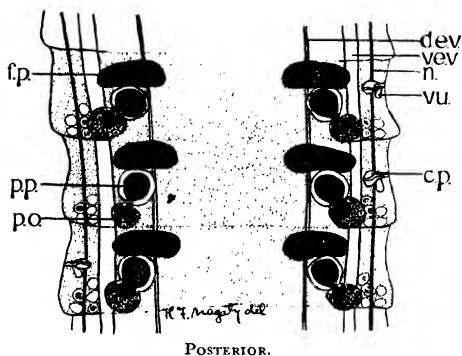


FIG. 5. *Stilesia globipunctata*. Partly gravid segments showing the shape of the fibrous structures in front of the paruterine organs, also the persistent testes and the dorsal excretory vessels. $\times 33$.

ANTERIOR.



POSTERIOR.

FIG. 6. *Stilesia globipunctata*. Gravid segments showing mature paruterine pouches, displaced paruterine organs, the opening between them, and the strongly-developed fibrous pads, and the still persistent dorsal excretory vessels. $\times 66$.

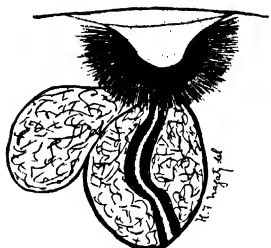
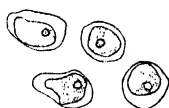
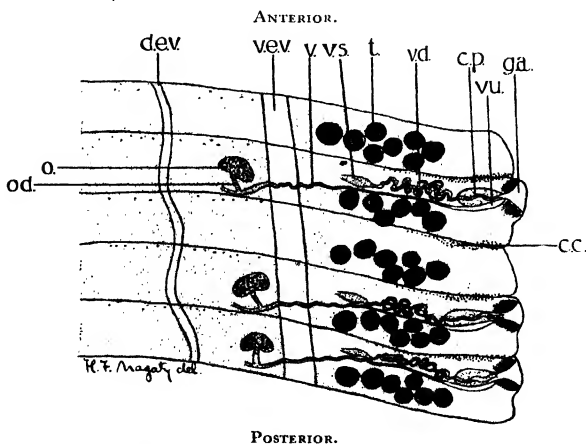


FIG. 7. *Stilesia globipunctata*. Showing the vulva, cirrus pouch and genital atrium surrounded by hair-like processes. $\times 400$.



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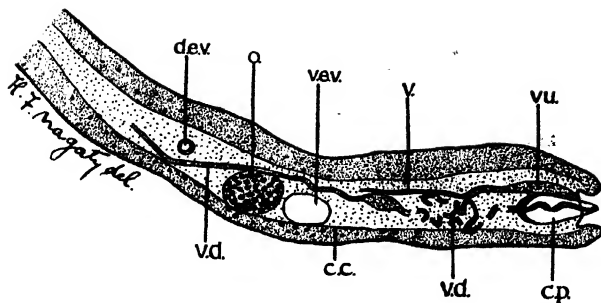
FIG. 8. *Stilesia globipunctata*. Showing variable shape of migrating ova. $\times 400$.



POSTERIOR.

FIG. 9. *Stilesia vittata*. Male mature segments showing the position of the calcareous corpuscles and the genitalia. $\times 60$.

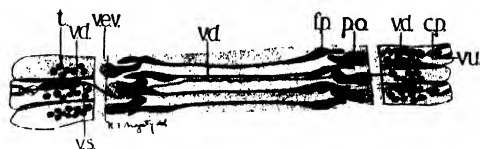
DORSAL.



VENTRAL.

FIG. 10. *Stilesia vittata*. Cross-section showing the relation of the cirrus pouches to the vulvae and the passage of the genital ducts between the excretory vessels. $\times 90$.

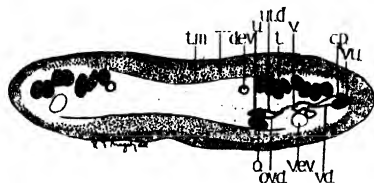
ANTERIOR.



POSTERIOR.

FIG. 11. *Stilesia vittata*. Gravid segments showing the complete course of the vas deferens—ovaries and dorsal excretory vessels have completely atrophied. $\times 30$.

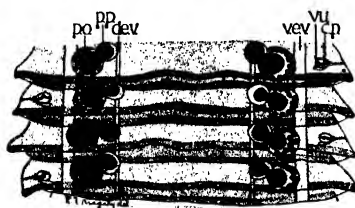
DORSAL.



VENTRAL.

FIG. 12. *Stilesia hepatica*. Cross-section in a male mature segment showing the relation of the genital ducts to the excretory vessels. $\times 30$.

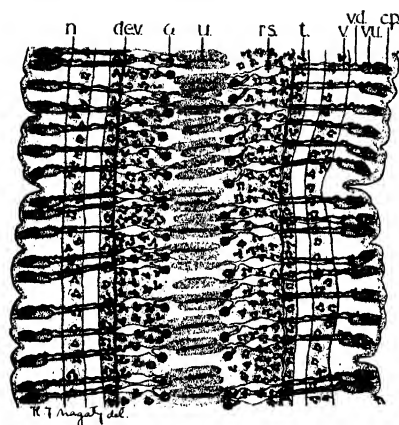
ANTERIOR.



POSTERIOR.

FIG. 13. *Stilesia hepatica*. Gravid segments showing the paruterine organs and pouches and the relations of the cirrus pouches to the vulvae. $\times 30$.

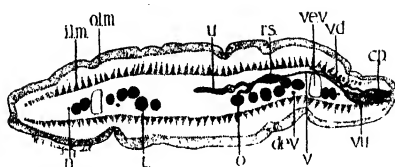
ANTERIOR.



POSTERIOR.

FIG. 14. *Avitellina centripunctata*. Showing male mature strobila. $\times 25$.

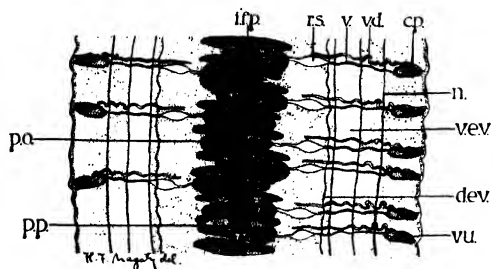
DORSAL.



VENTRAL.

FIG. 15. *Avitellina centripunctata*. Cross-section in a male mature strobila. $\times 30$.

ANTERIOR.



POSTERIOR.

FIG. 16. *Avitellina centripunctata*. Showing a gravid strobila. $\times 25$.

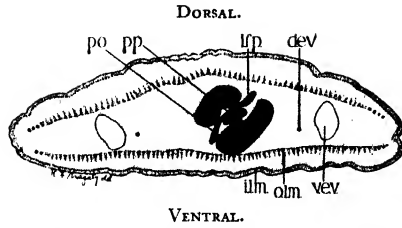


FIG. 17. *Avitellina centripunctata*. Cross-section in the gravid region. $\times 30$.

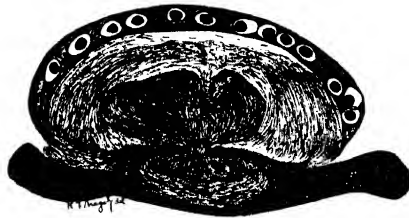


FIG. 18. *Avitellina centripunctata*. Showing paruterine organ and pouch and an intersegmental pad. $\times 125$.

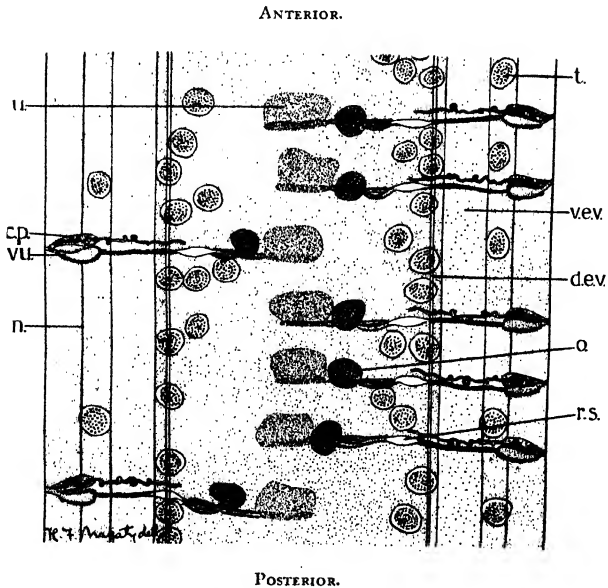


FIG. 19. *Avitellina sudanca*. Showing male mature strobila. $\times 30$.

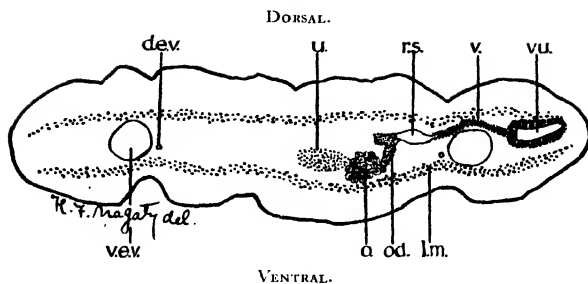


FIG. 20. *Aitellina sudanea*. Cross-section in the male mature strobila. $\times 90$.

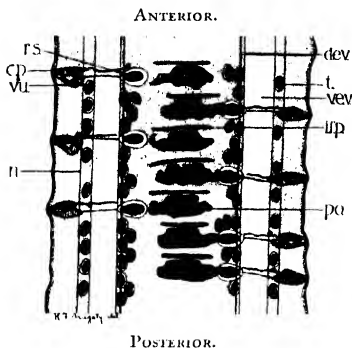


FIG. 21. *Aitellina sudanea*. Gravid strobila showing the enlarged receptacula seminis and the shape of the paruterine organs. The ovary have completely atrophied in this stage. $\times 30$.

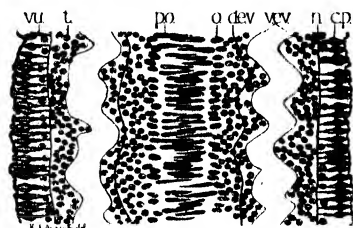


FIG. 22. *Aitellina southwelli*, n.sp. Showing male mature strobila. $\times 17$.

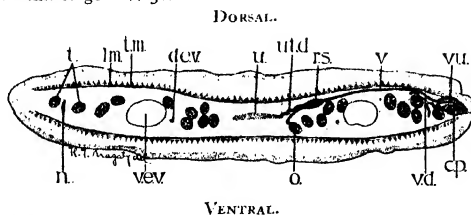


FIG. 23. *Aitellina southwelli*, n.sp. Cross-section in the male mature strobila. $\times 25$.

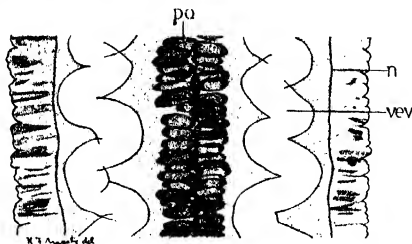


FIG. 24. *Aitellina southwelli*, n.sp. Showing the partly gravid strobila. $\times 17$.

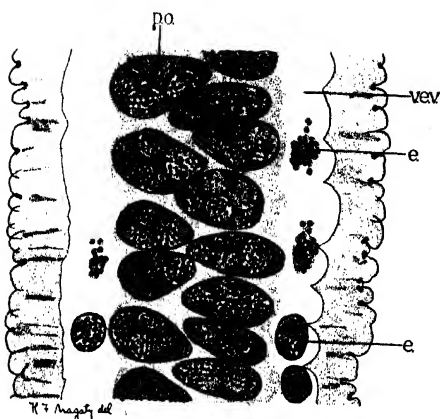


FIG. 25. *Avitellina southwelli*, n.sp. Showing the gravid strobila. $\times 25$.

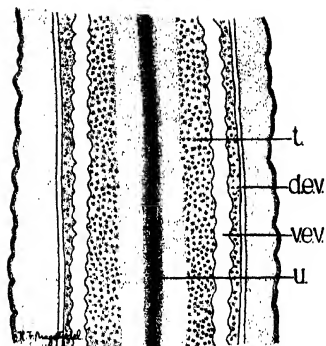


FIG. 26. *Avitellina aegyptiaca*, n.sp. Showing the immature strobila. The testes are beginning to develop. $\times 30$.

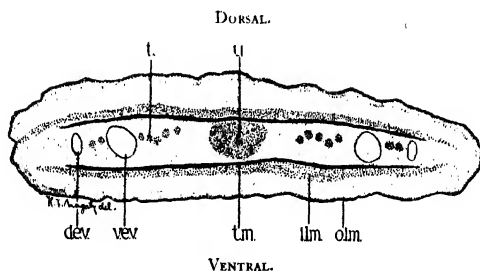


FIG. 27. *Avitellina aegyptiaca*, n.sp. Cross-section in the immature region. $\times 48$.

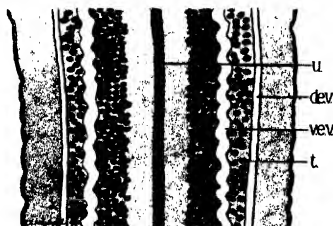


FIG. 28. *Avitellina aegyptiaca*, n.sp. Showing the male mature strobila. $\times 32$.

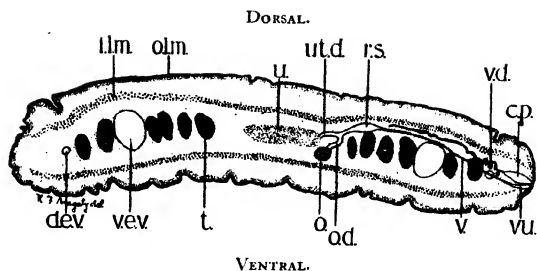


FIG. 29. *Avitellina aegyptiaca*, n.sp. Showing the cross-section in the male mature strobila. $\times 48$.

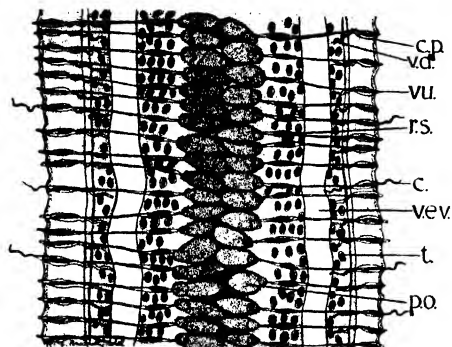


FIG. 30. *Avitellina aegyptiaca*, n.sp. Showing partly gravid strobila. $\times 30$.

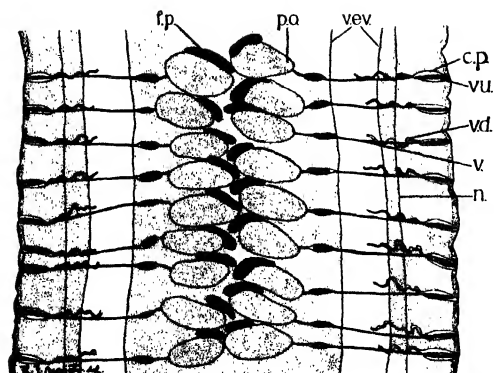


FIG. 31. *Avitellina aegyptiaca*, n.sp. Showing gravid strobila. $\times 48$.

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EXPLANATION OF PLATE V.

Stilesia globipunctata. Photograph showing the heads of the worms deeply embedded in the mucosa of the small intestine of sheep. $\times 2$.



Photograph by Miss M. E. Brown

ON A NEW SPECIES OF
PHYLLOBOTHRIUM (*P. MICROSOMUM*)
FROM AN INDIAN SHARK

BY
T. SOUTHWELL
AND
I. S. HILMY

(Received for publication 22 July, 1929)

Four specimens of this cestode have been obtained from the intestine of *Ginglymostoma concolor*. Pearl Banks, Ceylon. Pearson. No. 158. 9.3.21.

The worms are very minute and measure from 2.2 mm. to 2.4 mm. in length; they are composed of six or seven segments, the last one being nearly as long as the rest of the worm, and measuring about 1 mm. in length. The maximum breadth of the worm varies

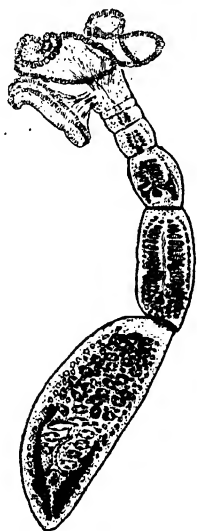


FIG. 1. *Phyllobothrium microsomum*. Entire worm. $\times 38$. Original.

from 234μ to 312μ . The genital pores are difficult to locate; they are irregularly alternate and situated a little behind the middle of the lateral margin of the segment. The head consists of four unarmed, boat-shaped bothridia borne on short stalks, each having a length of about 350μ and a breadth of 200μ . Their margins are definitely thickened, the rim having a breadth of about 17μ ; their shape and appearance vary considerably. Accessory suckers are absent, as is also a myzorhynchus. There is no neck.

Owing to lack of material nothing is known regarding the excretory, muscular and nervous systems.

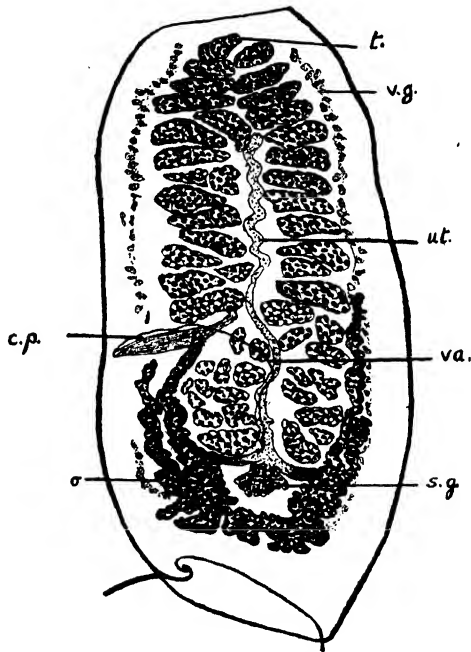


FIG. 2. *Phyllobotrium microsomum*. Penultimate segment. $\times 192$. c.p.—cirrus pouch; o.—ovary; s.g.—shell gland; t.—testes; ut.—uterus; va.—vagina; v.g.—vitelline glands. Original.

Testes. These first appear in the second or third segment and reach their maximum development in the last; their disposition in the latter is very different from that in the other segments; thus, in the penultimate one they are arranged in capsules, these being disposed in two longitudinal rows, one on each side of the median

axis. There are about twenty-five capsules on each side; they lie with their longer diameter transversely, and each measures about 50μ by 16μ . In the last segment the capsules have disappeared entirely and the testes lie free, occupying the greater part of the segment, the bi-lateral arrangement having been lost.

The writers wish to point out that this condition obtains in quite a number of species of Tetraphyllidea but has hitherto not been described.

The cirrus sac in the penultimate segment extends about a third the distance across, its internal extremity being directed anteriorly. The vas deferens is only slightly coiled and is situated close to the medial extremity of the sac.

The ovary is U-shaped, each limb being bifid; the aporal limbs are slightly longer than the poral and extend along the lateral margin almost to the middle of the segment.

The vagina is a simple thick-walled tube which, from the pore, runs anteriorly to the cirrus sac. The vitelline glands consist of two rows of acini, one running along, and close to, each lateral margin of the segment.

The shell gland is prominent and situated in the concavity of the ovary; it measures 25μ by 31μ . Immediately anterior to it can be seen the two oviducts, one from each ovarian limb, which meet in the middle line. They are continuous with the vagina and the uterus.

Uterus. Only the rudiments of this organ could be seen and this consisted of a granular condensation resembling a tube running along the median longitudinal axis.

As in practically all other species of this family, gravid segments and eggs are unknown. The species differs from others hitherto described in size and in being composed, when fully matured, of only about six proglottides.

On account of its small size it has been given the specific name of *microsommum*.

BOTHRIOCEPHALUS SCORPII

(MUELLER, 1776) COOPER, 1917

BY

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Synonymy extensive including the following :—

Vermis multimembris rhombi Leeuwenhoek, 1722.

Taenia scorpii Mueller, 1776.

Alyselminthus bipunctatus Zeder, 1800.

Taenia punctata Rudolphi, 1802.

Bothriocephalus punctatus Rudolphi, 1810.

The specimens to be described were obtained from the National Museum of Wales, bottle 28, 25.7.9, dated May 6th, 1928, collected from a turbot 3.5 miles south of Penkylan Head, Wales. They were preserved in 5 per cent. formaldehyde. The longest incomplete worm, still attached to the intestine, measured 26.5 cm. by 5 mm., and the smallest immature worm 1.25 cm. Many strobila with their scoleces, both gravid and immature, were found free in the bottle. They measured as follows :—

	Smallest.	Largest.
(1) Immature fragments with scolex... ..	9.5 mm. by 1.6 mm.	5.1 cm. by 2.1 mm.
(2) Immature fragments without scolex	1.9 cm. by 1.6 mm.	5 cm. by 2.1 mm.
(3) With scolex and gravid segments	About 7.15 cm. by 8.5 mm.	
(4) Same without scolex	2.5 cm. by 7.4 mm.	6.7 cm. by 4.8 mm.
(5) Fragments consisting of gravid segments only ...	1.3 cm. by 4.2 mm.	14 cm. by 6 mm.

No mature segments either with or without scoleces were found free in the bottle.

The colour of the preserved material is greyish-white. Crenulation of the lateral margins is just visible to the naked eye.

The scolex measures from 1.56 to 2.75 mm. in length by 940μ to 1.09 mm. in breadth by about 780μ in thickness. It is variable in shape in the preserved material. Its greatest breadth is found near the middle. The anterior extremity is sometimes as broad as the posterior, but it may be either broader or narrower, and the same applies to the thickness. The scolex terminates anteriorly in a square disc the face of which is usually a little concave but it may be convex. A slight to moderate constriction situated behind the terminal disc is always present. At the posterior third of the scolex there is often a transverse groove, not well marked. This is believed to be the beginning of the strobila.

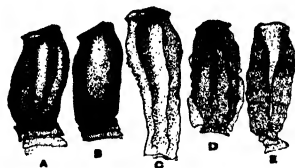


Fig. 1. *Botriocephalus scorpii*. Heads. A, B, C, and E—surficial view; D—lateral view. $\times 8$. Original.

Each of the two shallow bothria occupies the whole length of the scolex; they are very variable in shape. Some are broadest at their anterior extremity and assume the shape of a V or Y. They are usually deepest at about one-eighth the length of the scolex from the anterior extremity. From this point they gradually become shallower posteriorly, until the concavity has disappeared. In some specimens the sinuous margins of the bothria were seen in lateral view.

The strobila begins immediately behind the scolex. The first segment measures about 428μ in length by 624μ in breadth and 312μ in depth and it overlaps the anterior border of the second segment. In the latter careful examination shows a shallow furrow situated near the middle of each lateral margin. These become more marked in the third and fourth segments, giving each of them the appearance of the so-called double-segment. The latter increase gradually in length and breadth. At about 2 cm. from the anterior

extremity each segment giving rise to the 'double-segment' again shows indications of secondary shallow furrows. Whilst these furrows become increasingly marked posteriorly, the 'double-segment' divides into two segments each of which is likewise double, the anterior overlapping the posterior one. This process appears to be repeated several times, and takes place quickly until mature 'double-segments,' with 3, 4, 5, or even 6 sets of genital organs, are seen.

The formation of the secondary and tertiary furrows is apparently either delayed, or does not take place at all in the posterior one of the 'double-segments,' the anterior thus exercising a predominance over the posterior segment.

The genital rudiments appear in whole mounts at about 1.6 cm. behind the head whilst mature segments occur at about 3.3 to 5 cm. from the anterior extremity. They measure 624μ in length by 3.8 mm. in breadth.

Dark patches occur on one surface posteriorly; these represent the uterine sac filled with eggs. They alternate irregularly from one side of the mid-line to the other, and are situated near the anterior margin of the segment, but they may occur in the mid-line. On the opposite surface the genital pores can be made out in the unstained condition with a hand lens, as very minute, dark spots situated in a median, discontinuous groove.

The cuticula is 6μ in thickness and is formed of two layers; the outer, which stains more deeply, is composed of stout, closely-set pseudo-cilia; the internal is homogenous. Immediately beneath the latter is a very thin, sinuous, refractile membrane.

About 17μ internally to the latter membrane there is a layer of matrix cells arranged radially and irregularly at different levels and having a thickness of about 18μ . A considerable number of them is also found in other parts of the cortex and in the medulla.

In some specimens calcareous corpuscles measuring up to 18μ in diameter occur in abundance in the parenchyma.

The muscular system is composed of longitudinal, transverse, and dorso-ventral muscle fibres. The longitudinal fibres are comparatively abundant and form two layers, one external and one internal. The external layer is made up of a few bundles situated just beneath the cuticula. It can only be detected under

high-power magnifications and special staining methods. The internal layer occupies most of the space between vitelline glands and medulla and consists of large numbers of bundles which vary in thickness, those nearer to the vitelline glands are considerably stronger than those near the medulla. The transverse fibres are usually feeble and are situated between the internal, longitudinal muscles and the medulla, thus forming a sac which encloses the latter.

These transverse fibres are found to be slightly stronger between the 'double-segments,' evidence that internal segmentation takes place. The dorso-ventral muscles are found in the medulla only, especially in the lateral fields occupied by the testes and enclosing the latter.

There are two longitudinal nerves, one on each side of the median axis. Each nerve has a diameter of about 35μ and is situated at equal distances from both surfaces and at about one-fifth the breadth from the lateral margin. They appear in cross-sections as semi-circular, transparent structures and are surrounded, especially laterally, by few cells, some of which may be ganglion cells.

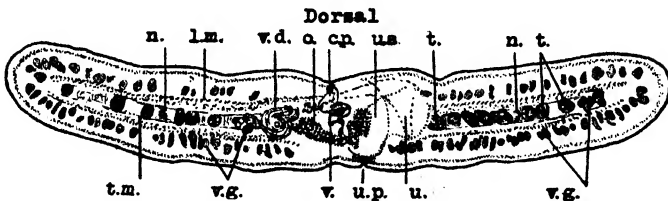


Fig. 2. *Bothrioccephalus scorpii*. Cross-section. $\times 25$. Original.

The excretory system shows great variations. In the youngest immature segments six longitudinal vessels, situated in the medulla can be seen, whilst in slightly older, mature and gravid segments they are very difficult to find. It appears as if they break up very early into branches of different dimensions which frequently communicate together by anastomoses. In one longitudinal, dorso-ventral (sagittal) section and at about one-quarter the breadth from the lateral margin, a longitudinal canal, measuring 10μ in diameter and following a sinuous course, is seen in the medullary parenchyma and is believed to be one of the main excretory vessels.

In immature segments the parenchyma is occupied by longitudinal muscles and special distinctive cells from which the genital organs develop. At first, a longitudinal, transversal field of genital cells which develop very rapidly is formed. All genital organs with the exception of the vitelline glands are derived from these cells. Another mass of special cells, from which the vitelline glands develop, appears later beneath the cuticula.

The first genital organs to develop are the testes. They are situated in a single layer in the medullary parenchyma forming two longitudinal fields, one on each side of the median axis. They are continuous from segment to segment and do not extend in front of or behind the ovary. Each field measures 870μ in breadth in a gravid segment, 3.6 mm. in breadth and is situated at equal distances from both surfaces and at about 300 to 350μ inwardly from the lateral margin of the segment. One set of genital organs comprises about 44 testes in each lateral field, but this number varies greatly. Their shape is subspherical (38μ by 42μ by 42μ) to ovoid (63μ by 28μ by 28μ); in the latter case the longer axis is directed transversely.

In gravid segments the testes shew all stages of development including primary testicular cells, morula-like cells, some with peripheral arrangements of the nuclei; elongated chromatin bodies; unripe spermatozoa and lastly fully developed ones. A thick tunica propria is present. The vasa efferentia are very difficult to make out. They are very fine and have a structureless wall.

The cirrus pouch measures about 122.5μ by 70μ and is broadest at about the junction of its middle and proximal third. It is ovoid in shape and is directed ventrally and slightly anteriorly. At about its distal fifth it is slightly bent towards that lateral half which contains only the smaller part of the uterine duct. This is invariably the case in mature and gravid segments. Its wall is thick and muscular and is covered on both surfaces with myoblastic nuclei. Few muscle fibres are arranged in an irregular manner inside the pouch.

At the proximal end of the cirrus pouch, immediately behind the opening of the vas deferens into it, and closely attached to its wall is a comparatively small vesicula seminalis interna which has a maximum diameter of about 10μ . It is pyriform in shape and has

its wider extremity situated proximally. Gradually it becomes narrow distally and at a distance of about 18μ from the proximal extremity of the sac its lumen becomes of the same diameter as that of the ductus ejaculatorius. The latter is much coiled in mature, less so in gravid, segments. Its lumen measures from about 2 to 2.5μ in diameter, and its wall is composed of two distinct layers, the inner of which stains more deeply and is continuous with the cuticula surrounding the very deep genital pore; its inner surface is not smooth but ragged and its outer wall is apparently muscular and does not extend beyond the cirrus pouch but is continuous with the wall of the latter.

From its proximal to its distal extremities the canal has the same structure and it is evident, therefore, that cirrus and ductus ejaculatorius are histologically not distinct.

The vas deferens is a mass of coils situated in the anterior part of the segment and laterally to the median axis. It occupies in gravid segments a space which measures 40μ in length, 225μ in breadth, and about one half the thickness of the segment. In mature segments its lumen is much narrower than in gravid ones. As the lumen becomes distended with spermatozoa it gradually becomes wider, the walls become attenuated and their cells atrophy leaving spare, oval shaped nuclei. The proximal parts of the vas deferens may attain a diameter of from 30 to 40μ . The distal parts are narrower (9μ), their walls are relatively much thicker, their nuclei more conspicuous and numerous. Before it enters the cirrus pouch the vas deferens has a lumen of about 7μ in diameter and is surrounded by a large number of prostatic cells situated for the most part anteriorly. There is no vesicula seminalis externa but in one of the segments the walls of the vas deferens could not be made out and the whole mass of spermatozoa appeared to be enclosed in a sac, whilst preceding and succeeding segments were as described above.

The vaginal pore is situated immediately behind and slightly laterally to the cirrus pouch.

The vagina begins as a very narrow duct, 2.5μ in diameter, proceeding towards the centre and just posteriorly to the cirrus pouch for a distance of about 45μ . In the succeeding 30μ of its length the lumen becomes gradually wider until it measures about 10μ in diameter, then it suddenly becomes as narrow as before at a point

75 μ from its distal extremity and remains so for the next 30 μ . It then becomes wider again as it proceeds towards the ovary. When it has traversed a total distance of about 150 μ in a dorso-ventral direction, and in a sinuous manner it makes a sharp curve posteriorly and then laterally for a distance of about 15 μ to approach the oviduct from a lateral point. From its distal to its proximal extremities the internal wall of the vagina retains its cuticular structure, but, unlike the cirrus, its inner surface is smooth. The outer wall is muscular. All along its course except in its narrower parts, the vagina is seen distended with spermatozoa.

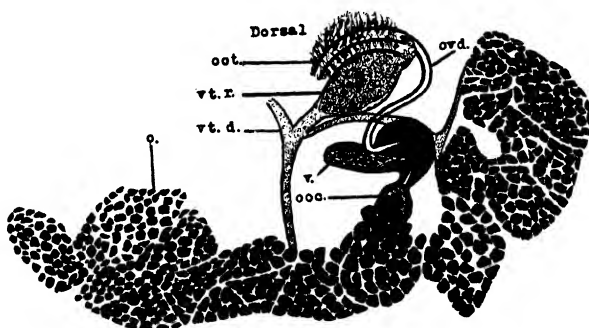


Fig. 3. *Bothrioccephalus scorpii*. Female genital organs viewed along the longitudinal axis of the segment. $\times 180$. Original.

The ovary is an antero-posteriorly flattened organ situated along the transverse axis and at the extreme posterior extremity of the segment. It measures about 112 μ in length, 350 to 450 μ in breadth and 50 μ in thickness. Its lateral parts are slightly thicker and curved towards the dorsal surface. The isthmus is broad and deep but it may rarely be shallow. It is convex towards the ventral surface. The fact that the isthmus is nearer to one surface (120 μ) than to the other is the only indication as to which is the ventral and which the dorsal surface of the two. At about its middle and protruding towards the centre the isthmus is prolonged into a mass of cells which here attain their maximum dimension, and are without nuclei. One or two of these cells are seen in the lumen of the oocapt. This is funnel-shaped, measuring 33 μ in diameter and is covered with well-developed circular muscle fibres. Its axis is directed posteriorly, slightly laterally, and, in some cases, slightly dorsally; its narrow

extremity opens into the oviduct. The latter begins as a wide sac into which the vagina opens as stated above, and which measures 33μ by 50μ by 37μ , including its very thick muscular walls. On the dorsal aspect of this sac the oviduct, which has a lumen of 8μ in diameter and a wall 3μ in thickness, proceeds for a short distance in a dorsal direction; it then partly encircles the yolk reservoir. Immediately after it has received the common yolk duct from the latter it becomes continuous with the ootype. This is situated just dorsally to the yolk reservoir, to the dorsal margin of which it runs parallel, that is, laterally and slightly anteriorly. Its lumen measures 8μ and its wall is surrounded by the voluminous shell gland for a distance of 120μ .

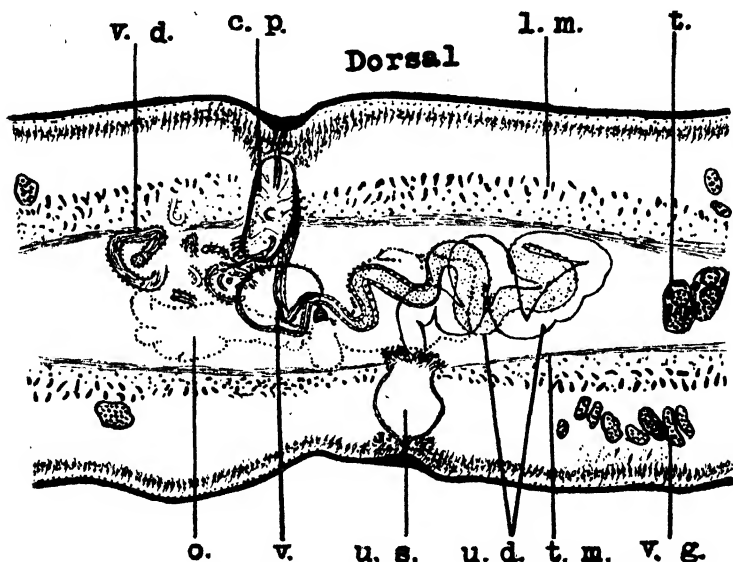


Fig. 4. *Botriocephalus scorpii*. Uterus. $\times 120$. Original.

The continuation of the ootype is the uterus. It begins as a very narrow duct which becomes gradually wider as it proceeds distally. In mature segments it forms two sinuous, transverse layers. In cross sections the uterus can be followed easily, whilst in whole mounts it is viewed along its longitudinal axis and it is, therefore, seen as small circular structures which may be mistaken for unripe eggs.

The first layer is situated posteriorly and extends from the ootype laterally towards the ventral surface, thereby crossing the ovarian lobe anteriorly. From here, in a mature segment 3.6 mm. in breadth the uterus crosses the whole breadth of the ovary anteriorly towards the other side of the median axis for a distance of 350μ ; it then bends sharply to form the anterior layer. Its lumen has now a diameter of about 45μ ; after a slightly sinuous course it becomes narrower during its last curve, and approaches the uterine sac. The latter is situated near the ventral surface, very often laterally, but in some cases in the median plane. It is globular at first becoming conical as the segments mature, until in gravid ones it extends nearly throughout the whole depth of the segment; its longest axis is directed dorsally and slightly laterally, and posteriorly. In mature segments it measures 75μ in its greatest breadth and 112μ in depth; in gravid segments the axis of the cone measures 245μ and the ventrally situated base 100μ in length by 175μ in breadth.

The walls of the uterus, which are lined with epithelial cells, become gradually attenuated and the cells atrophy as the lumen becomes wider in order to accommodate the developing eggs. In mature segments the junction between the uterine duct and the sac is obliterated by a large mass of cells, the nature of which is believed by some helminthologists to be glandular so as to facilitate the passage of the eggs to the exterior. As mentioned above the uterine sac becomes wider as it develops; the obliteration between the uterine duct and the sac becomes less marked, disappearing later, and in the meantime a slit-like opening appears between the sac and the exterior.

The vitelline glands are the last to develop. They occupy two lateral fields in the cortical parenchyma, and are occasionally represented towards the mid line by a few follicles. In addition to this, and more frequently, a few follicles are present in the medullary parenchyma between the testes. This confirms the statement by Matz that one follicle may be found in some cases near the lateral border of the ovary. They are ovoid in shape and measure about 35μ by 30μ by 70μ . The vitelline ducts are quite variable in diameter in different segments; in some cases they are wide and are often found distended with yolk cells; in others they are very narrow and are difficult to follow. They tend to anastomose

dorsally and ventrally. From each surface two ducts, one on each side of the mid-line, arise and run towards the medulla. Near the transverse axis, each duct meets the other from the opposite surface. From each of these dorso-ventral ducts another arises, one of which is short and wide and forms the vitelline reservoir, when this is present; the other opens into the first. The vitelline reservoir

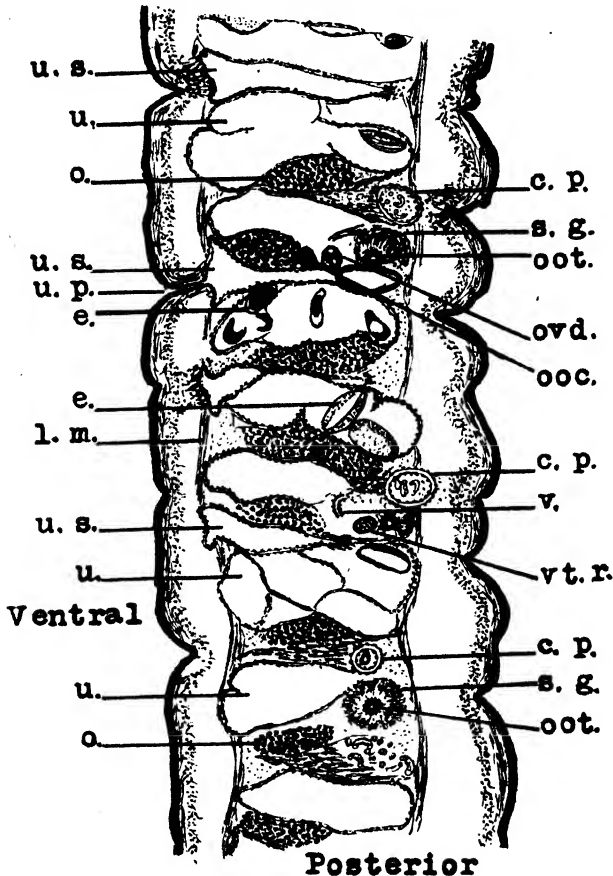


Fig. 5. *Botbriocephalus scorpii*. Sagittal section. $\times 90$. Original.

measures 40μ by 60μ by 50μ and is ovoid in shape; it is situated dorsally to the ovary, its longest axis being nearly parallel to the transverse axis of the segment. One pole, which receives the yolk cells, is directed posteriorly and dorsally. From the other the common

vitelline duct arises. This has a diameter of 9μ and is short. It opens into the oviduct just before this becomes continuous with the ootype. In specimens with very wide peripheral vitelline ducts no vitelline reservoir is usually found, but instead, two wide central ducts, forming a **U** with both limbs nearly parallel to the transverse axis, one being shorter than the other. The common vitelline duct arises from the convexity.

The shell gland is a voluminous organ measuring 90μ by 120μ by 90μ , situated posteriorly and dorsally. It is best seen in sagittal sections where it is found to encircle the ootype, there being a clear space between the latter and the gland. Processes from the gland cells cross this space thus placing the gland in communication with the ootype.

The eggs in utero are ovoid in shape and measure 80μ by 45μ by 60μ . They are dark grey in colour and contain what appears to be a very small ovum, this being surrounded by a dense mass of yolk cells, and the whole being enclosed in a transparent envelope. The egg-shell has a thickness of 1μ only. Under the oil-immersion lens what is believed to be a very narrow operculum has been observed in few cases at one pole. This evidence was supported by the fact that when the eggs were pressed under a cover-slip and thus ruptured they almost invariably did so in a position which would normally be occupied by an operculum. In some cases the operculum was seen still hanging to the ruptured egg and it measured 23μ in breadth.

The writer wishes to express his indebtedness to Dr. T. Southwell, of the Liverpool School of Tropical Medicine, for his valuable advice and help.

ABBREVIATIONS

<i>c.p.</i>	= cirrus pouch.
<i>e.</i>	= eggs.
<i>l.m.</i>	= longitudinal muscles.
<i>o.</i>	= ovary.
<i>ooc.</i>	= oocapt.
<i>oot.</i>	= ootype.
<i>ovd.</i>	= oviduct.
<i>s.g.</i>	= shell-gland.
<i>t.</i>	= testes.
<i>t.m.</i>	= transverse muscles.

<i>u.</i>	= uterus.
<i>u.d.</i>	= uterine duct.
<i>u.p.</i>	= uterine pore.
<i>u.s.</i>	= uterine sac.
<i>v.</i>	= vagina.
<i>v.d.</i>	= vas deferens.
<i>v.g.</i>	= vitelline gland.
<i>vt.d.</i>	= vitelline duct.
<i>vt.r.</i>	= vitelline reservoir.

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JARDUGIA PARADOXA, A NEW GENUS AND SPECIES OF CESTODE WITH SOME NOTES ON THE FAMILIES ACOLEIDÆ AND DIPLOPOSTHIDÆ

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Southwell, in 1926, gave a short description of a worm which, he pointed out, was of doubtful systematic position, but which, in certain anatomical features, resembled *Diploposthe lævis*. His account was as follows :—

‘ One specimen from a small heron (*Ardea* sp.). Azare, N.P. Nigeria, 20.viii.25. Collected and presented by Dr. I.I. Lloyd.

‘ The specimen was peculiar in that in the anterior half of the strobila only a single set of male genital organs were present and these were unilateral. About the middle of the length of the worm the second set of male genitalia appeared suddenly, but irregularly. Posteriorly a double set of male genitalia was present in all segments except in four, which bore a single set.

‘ The female genital organs appeared about the middle of the length of the worm : they were normal, except that in a few of the most posterior segments portions of the ovary had not atrophied and they were a conspicuous feature of segments in which otherwise only the cirrus pouches and uterus were visible.’

A full description of this unique parasite, which is now considered as the type-species of a new genus, is given below :—

The length of the worm is approximately 8.5 cm. and the greatest breadth 2.35 mm. For the purpose of examination it was cut into four fragments.

The scolex measures 333μ in breadth. It is pear-shaped and is provided with four unarmed, cup-shaped suckers, each of which has a diameter of 130μ . The invaginated rostellum, which is pointed at its anterior extremity, measures 244μ by 88μ , and is armed with a

single crown of about ten hooks, each of which measures from 13 to 18 μ in length.

The first signs of segmentation are visible about 800 μ from the anterior extremity. Here the breadth of the worm is 1.6 mm.

The cuticula measures 2 μ in thickness. It is composed of three distinct layers, the external and internal of which are membranous and stain deeper than the intermediate, homogenous and comparatively thicker layer. The matrix cells are situated immediately beneath the cuticula and have a thickness of about 15 μ . The cells are spindle-shaped with small, round nuclei.

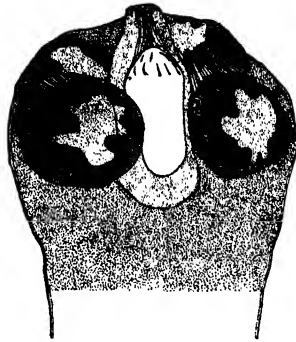


FIG. 1. *Jardugia paradoxa*. Head. $\times 116$.

There are three layers of longitudinal muscles. The external layer is situated between the cuticula and the matrix, and is composed of single bundles 3 μ in diameter and placed at distances of about 7 μ from each other. The intermediate layer lies about 40 μ from the cuticula inwards, and is composed of groups of stouter bundles; each group measures about 15 μ in thickness, and occurs at distances of from 15 to 40 μ from one another. The internal longitudinal layer is the most strongly developed. It is situated about 75 μ inwards from the cuticula. The single bundles may attain a thickness of 15 μ and are arranged in groups which may measure 35 μ or more in thickness.

There are two longitudinal sinuous excretory vessels in each lateral half. The larger vessel is situated internally to the smaller and at the junction of the lateral and middle quarters of the segment. It measures from 50 to 70 μ in diameter and lies at a distance of

about 140μ from the ventral surface, where the thickness of the segment is about 420μ . The smaller vessel, which measures 4μ only in diameter, is situated at about 77μ laterally and 24μ dorsally to the ventral vessel.

Calcareous corpuscles are abundant and are found mainly in the peripheral parts of the parenchyma; they attain a maximum diameter of about 12μ .

The most striking feature of this worm is the arrangement of the genital organs. In the first fragment, which measures 1.75 cm. in length, the genital rudiments appear, in whole mounts, at about 1.6 mm. from the anterior extremity, and are at first situated in the mid-line, but soon become confined to one lateral half, viz., the right

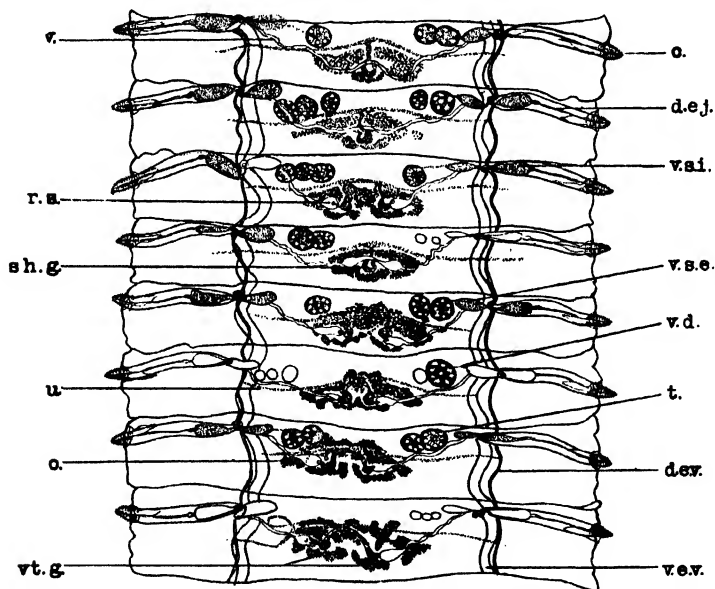


FIG. 2. *Jardugia paradoxa*. Eight segments with mature male genitalia. Dorsal view. $\times 26$.

half. At 4.5 mm. from the anterior extremity these rudiments become distinctly elongated transversely, the elongations forming the male genital ducts, whilst the testes differentiate from the medial parts. In about the 130th segment, which is situated 1.6 cm. from the anterior extremity, these genital structures become bilateral owing to the striking and sudden appearance of male organs in the

left half of the worm where, previously, no rudiments have been visible, the only difference being that two testes only are found in this half against three in the opposite half. The succeeding segment (the 131st) possessed one set only; the second segment (the 132nd) had double sets and the remaining few segments (the 133rd to the 142nd) had again each one set.

In the second fragment, which measures 3 cm. by 2.3 mm., the male genital organs have attained their maximum development. The first nineteen segments possess one set of male genital organs in the right half. Then there were :—

Two male sets in the 20th ;

One male set in the 21st to 26th ;

Two male sets in the 27th, and

One male set in the 28th and 29th.

From the 30th to the 89th segment (the last in the second fragment) the male genitalia were double, as was observed in the remaining two fragments of the worm.

The testes are globular in shape and measure from 40μ when they first appear, to 123μ in mature segments. They are situated in front of the ovary, in the anterior half of the segment and near the dorsal surface; they lie parallel to the transverse axis of the segment and laterally to the median axis. In segments possessing only one set of genital organs, there are usually three testes confined to the corresponding half (the right half); two segments, however, possessed two testes only. In the other segments with double male genitalia, there are usually three testes in the right half and two in the left half, but the number on each side varies between none and four. Altogether there are from two to six testes in each segment. They soon begin to atrophy and sometimes disappear before the female genital organs have attained maturity.

The vas deferens is short and inconspicuous. It arises anteriorly to the testes and proceeds towards the lateral margin; it very rapidly widens into a vesicula seminalis externa. This conspicuous organ, which is oval (seldom globular) in shape, is situated in line with the cirrus pouch and at its proximal extremity; it is very variable in size, the largest measuring 230μ by 140μ . It is generally found distended with spermatozoa. Its lateral (distal) termination extends beyond the proximal extremity of the pouch and remains

dorsally to the latter for a short distance ; it then narrows suddenly and, turning in the medial direction for a short distance, eventually forms a loop before it enters into the pouch. The latter organ is an almost cylindrical sac with its longitudinal axis directed transversely ; it measures from 380 to 625μ by 70 to 85μ , and is situated in the anterior half of the segment and near the dorsal surface ; it is either straight, curved or sinuous, but in each case its distal extremity is nearly always directed slightly posteriorly ; its proximal extremity is at about the same position as the excretory vessels which the pouch passes dorsally.

Just after the male genital duct enters into the pouch it widens again into an internal vesicula seminalis. This is elongated and measures up to 210μ ; it is often as broad as the pouch itself, being broadest at its distal extremity, where it narrows suddenly and becomes continuous with the ductus ejaculatorius. The latter is an almost straight duct when the cirrus is protruded ; it measures 175μ by 10μ ; its proximal extremity has a wide lumen (17μ) and is provided with a strong sphincter muscle.

The cirrus, which in mature segments nearly always protrudes about 85μ , measures 210μ in length. Its protruded part is covered with fine, closely-set spines, each of which measures about 3μ , and which have a linear arrangement. The junction of cirrus and ductus ejaculatorius, within the cirrus pouch, is surrounded by a dense mass of prostatic cells.

The genital sinus, which has a diameter of 70μ , rarely 100μ , and a breadth of 52μ , is situated at about the junction of the anterior and middle thirds of the lateral margin of the segment.

The vagina lies ventrally to the cirrus pouch ; it begins with a diameter of 24μ and, as it proceeds inwardly and parallel to the cirrus pouch, its lumen narrows gradually over a length of 100μ until it has a diameter of 7μ . Its diameter then varies between 7μ and 15μ until it has passed, together with the cirrus pouch, dorsally to the longitudinal excretory vessels. When it has thus covered a total distance of 625μ or, at the junction of the lateral and middle quarters of the segment, it turns suddenly and, for a very short distance, dorsally ; later, with a distinctly wider lumen but of variable size, it follows a sinuous course posteriorly and medially towards the ovary, thus passing posteriorly and eventually ventrally to the testes. At

its proximal (central) extremity the lumen becomes distinctly wider and an oval to pyriform receptaculum seminis is formed which may measure up to 60μ by 90μ .

As in the male, so in the female, the duplication is at first irregular; thus in the second fragment, above-mentioned, segments Nos. 1 to 25, 27 to 49, 52 to 54, 58 to 65 and, lastly, 67, possess only one set of female organs with the exception of the vagina which, where double male genitalia occur, is also doubled, in which case it always communicates with the vagina of the right half, thus forming a transverse arch across, and dorsally to, the female organs. In these cases also, there may be one receptaculum seminis or two, but, more frequently, this organ is absent.

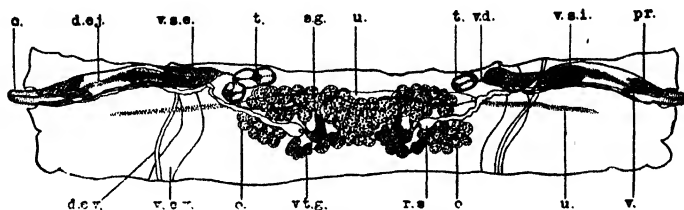


FIG. 3. *Jardugia paradoxa*. Segment with mature female genitalia. The testes are almost atrophied. Dorsal view. $\times 36$.

It should be noted, firstly, that in segment No. 19 (of the second fragment) which, as mentioned above, possesses two sets of male and one set of female genital organs, the left vagina extended only to the point where it usually, when mature, changes its transverse course into a posterior and medial one to approach the ovary; secondly, in segment No. 50, which, as mentioned above, possesses two sets of both male and female organs, the same was observed with the left vagina, the unusually wide proximal end of which apparently ends blindly and has a diameter of 44μ .

The ovary appears at about 9.6 mm. as a small collection of cells situated posteriorly to the testes and near the median axis. This mass of cells soon increases in size and elongates transversely; it then becomes slightly curved antero-posteriorly, the concavity being directed posteriorly. The ovary continues to increase in size in the posterior segments until it occupies the middle third of

the breadth and more than three-quarters of the length of the segment. Here the single ovarian follicles are globular and have a diameter of 70μ . They are very numerous and are arranged irregularly around the female ducts, the concavity being directed dorsally and posteriorly. Several attempts to determine the exact number of follicles present in each ovary failed, but they may number at least forty. In their last stage of development the ovaries cease to function and, later, disappear suddenly and irregularly, so that in some segments they are absent altogether whilst preceding and succeeding ones contain only traces of the organ.

The vitelline gland is the most posteriorly situated of all organs and is nearer to the dorsal surface than to the ventral. It is deeply lobed and occupies up to 70μ of the length and 160μ of the breadth of the segment. It discharges the yolk cells into the oviduct from a posterior and eventually dorsal point.

The oviduct is short and wide and arises from the dorsal aspect of the ovary; after receiving the yolk duct it becomes continuous with the ootype. This is very short and sinuous and is directed anteriorly to open, after passing through the inconspicuous shell gland, into the uterus. The ootype from each side may open separately into the uterus or each may proceed anteriorly and medially to meet the ootype of the opposite half, thus forming an arch from the convexity (anterior aspect) of which the uterus proceeds anteriorly for a very short distance (about 85μ).

The uterus is seen at first as a transverse line of cells penetrating in its middle parts between the ovarian follicles. It may extend, whilst still in this stage of development, beyond the longitudinal excretory vessels, which it crosses dorsally. When mature it becomes very wide and increases so much in length that it becomes sinuous to accommodate itself in the limited and even decreased breadth of the segment. No fully gravid segments were found and accordingly the eggs are unknown.

The new genus *Jardugia* is defined as follows:—

Scolex with an armed rostellum. Each segment of the strobila has a single or double set of male, situated in front of a single or double set of female genital organs. Uterus a transverse sinuous sac. Adults parasitic in birds.

Type-species :—*Jardugia paradoxa* n.sp.

Up to the present the following species of cestoda have been recorded from *Ardea* :—

Ardea cinerea Linn.

Dilepis campylancristrota (Wedl, 1855).

Acanthocirrus cheilancristrota (Wedl, 1855).

Hymenolepis microcephala (Rudolphi, 1819).

Tænia leuckarti Krabbe, 1869.

Ardea spp.

Tænia leuckarti Krabbe, 1869.

Anomotænia aurita (Rudolphi, 1819).

Dilepis hoplites (v. Linstow, 1903).

Systematic. The family DILEPIDIDÆ Railliet and Henry, 1909, is divided into the following three sub-families :—

(1) DILEPININÆ. Uterus persistent.

(2) DIPYLIDINÆ. Uterus replaced by egg capsules.

(3) PARUTERININÆ. Uterus developes paruterine organs.

It is clear that, as the uterus in our species is persistent, the only sub-family to which it might be referred is DILEPININÆ. But none of the genera in this sub-family is characterised by the possession of a double set of genital organs.

The characters of the family ACOLEIDÆ Ransom, 1909, are as follows :—

‘Tænioidea : Scolex generally armed, seldom without rostellum. Suckers unarmed. Strobila thick with short segments. Musculature consists of at least two layers of longitudinal muscles alternating with layers of transverse muscles. A single set, double set, or partial duplication of reproductive organs in each segment. Male genital openings marginal. Female genital (vaginal) opening lacking. Cirrus always very large and armed with strong hooks or spines. Eggs with thin transparent shells. Adults in birds.’

Type-genus :—*Acoleus* Fuhrmann, 1899.

It contains the following genera :—*Acoleus* Fuhrmann, 1899, *Diploposthe* Jacobi, 1896, *Gyrocelia* Fuhrmann, 1899, *Dioicocestus* Fuhrmann, 1900, *Diplophallus* Fuhrmann, 1900, *Shipleya* Fuhrmann, 1907, *Progynotænia* Fuhrmann, 1909, *Proterogynotænia* Fuhrmann, 1911, *Urocystidium* Beddard, 1912 and *Monoecocestus* Beddard, 1914, in all of which a vaginal pore is absent except in the genus *Diploposthe*.

Fuhrmann, in 1926, placed the genus *Diploposthe* in the family HYMENOLEPIDIDÆ on account of the fact that there are usually three, rarely seven, testes in each segment.

Poche, in the same year, created a new family DIPLOPOSTHIDÆ, to accommodate the genus *Diploposthe* which clearly could not be

retained in the family ACOLEIDÆ. He defined the family as follows :—

‘*Tæniinca* with flattened bodies, distinct internal and external segmentation, a rostellum with one crown of hooks, unarmed suckers, short proglottides which have no appendages. The musculature, from the exterior to the interior consisting of a weak diagonal muscle layer, a strong, circular muscle just in front of the posterior margin of the proglottis, a complete external and an incomplete internal layer of longitudinal muscles and a layer of transverse muscles. Double genital pores each situated at the lateral margin in a genital sinus; centrally situated genital glands of which the female are in front of the male; three to (?) seven testes; a vesicula seminalis, a strongly developed cirrus pouch and a cirrus armed with strong spines in each lateral half; a bilobed ovary in front of the vitelline gland; a simple, sinuous, transverse uterus which forms several huge diverticula; vagina double and opening ventrally to the cirrus; eggs, when ripe, have, in addition to the very thin, extensible and transparent egg-shell, two more thin coverings.’

Of the ten genera referred to in the family ACOLEIDÆ, three, viz., *Diploposthe*, *Diplophallus* and *Dioicocestus*, differ so widely from the rest that it appears desirable to separate them.

Southwell, in ‘The Cestode Fauna of British India’ (in the press) places the genus *Dioicocestus* in a new family which he names DIOICOCESTIDÆ, the character of which is that the sexes are separate.

In the genera *Diploposthe*, *Diplophallus* and *Jardugia* n., the genital organs show either complete or partial duplication. The following table will emphasize the relationship between them :—

	<i>Diploposthe</i>	<i>Diplophallus</i>	<i>Jardugia</i>
Male organs	Double in each segment	Double in each segment	Single or double in each segment
Number of testes in each segment ...	3 to 7	About 100	3 to 5
Position of testes ...	Posterior to ovary	In two lateral fields	Anterior to ovary
Female organs	Single in each segment but with two vaginæ	Single in each segment but with two vaginæ	Single or double with one or two vaginæ
Vaginal pore	Present	Absent	Present
Uterus	A transverse, sinuous sac	A transverse, sinuous sac	A transverse, sinuous sac

It will be seen that these three genera form such a natural group that, as they differ so widely from the remaining genera of the family ACOLEIDÆ, the writers propose placing them in the family DIPLOPOSTHIDÆ Poche, 1926, the definition of which is accordingly emended as follows :—

Head with an armed rostellum. Mature segments broader than long. Musculature well developed. A single or double set, or a partial duplication of male and female genital organs in each segment. Vaginal pore present or absent. Cirrus very large and armed with spines. Uterus a transverse, sinuous sac. Adults parasitic in birds.

Type-genus :—*Diploposthe* Jacobi, 1896.

It now becomes necessary to re-define the family ACOLEIDÆ Ransom, 1909, which is accordingly done as follows :—

Scolex usually armed. Musculature consists of at least two layers of longitudinal alternating with layers of transverse muscles, except in the genus *Monoecocetus*. A single set of genital organs in each segment. Vaginal pore absent. Adults parasitic in birds and mammals.

Type-genus :—*Acoleus* Fuhrmann, 1899.

EXPLANATION OF LETTERING.

<i>c.</i> = cirrus.	<i>t.</i> = testes.
<i>c.p.</i> = cirrus pouch.	<i>u.</i> = uterus.
<i>d.e.v.</i> = dorsal excretory vessel.	<i>v.</i> = vagina.
<i>d.e.j.</i> = ductus ejaculatorius.	<i>v.d.</i> = vas deferens.
<i>o.</i> = ovary.	<i>v.e.v.</i> = ventral excretory vessel.
<i>pr.</i> = prostatic cells.	<i>v.s.e.</i> = vesicula seminalis externa.
<i>r.s.</i> = receptaculum seminis.	<i>v.s.i.</i> = vesicula seminalis interna.
<i>sb.g.</i> = shell gland.	<i>vt.g.</i> = vitelline gland.

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DESCRIPTIONS OF THE EARLY STAGES OF TWO FURTHER MOSQUITOS COLLECTED IN SOUTHERN NIGERIA BY MR. L. H. DUNN

BY

A. M. EVANS

(Received for publication 14 October, 1929)

In a previous paper I described the larva and pupa of *Aedes* (*Armigeres*) *albomarginata* var. *dunni* taken by Mr. L. H. Dunn, in Lagos, when a member of the West African Yellow Fever Commission in 1927. The two larvae and pupae which form the subject of this paper were also collected and reared to the adult stage, in and near Lagos, by Mr. Dunn, to whom I am greatly indebted for the privilege of describing them.

HARPAGOMYIA FARQUHARSONI Edw.

The larva and pupa of this myrmecophilous species have many features in common with those of *H. genurostris* (*splendens*) described by de Meijere (1911), and *H. trichorostris*, recently described by Ingram and Meillon (1927).

Larva (fig. 1, A-D). The shape of the head resembles that of *H. genurostris* and *H. trichorostris*, being rounded in front and widest along the posterior border. The clypeal hairs (*cl.*) are multiple tufts of subplumose branches and project well beyond the anterior border of the head. The inner anterior are composed of 7 to 8 branches; outer anterior 5 to 7; posterior 2 or 3. The dorsal hair rising in front of the base of the anterior is 7 to 9 branched. Frontal hairs are not present in the normal position but, internal to the eye are two very delicate, branched hairs. From the lateral margin a small branched hair projects and ventro-laterally are three tufts, the anterior of about 10, the mid 4 and the posterior 6 branches. Small palmate tufts of 4 stout bristles lie posteriorly on the ventral surface. The antennae are small, slightly curved and

narrowing distally with a double hair on the distal half. Mental plate, as in *H. trichorostris*, very narrow from before backwards, with 12 teeth (11 well developed), on each side of the central tooth.

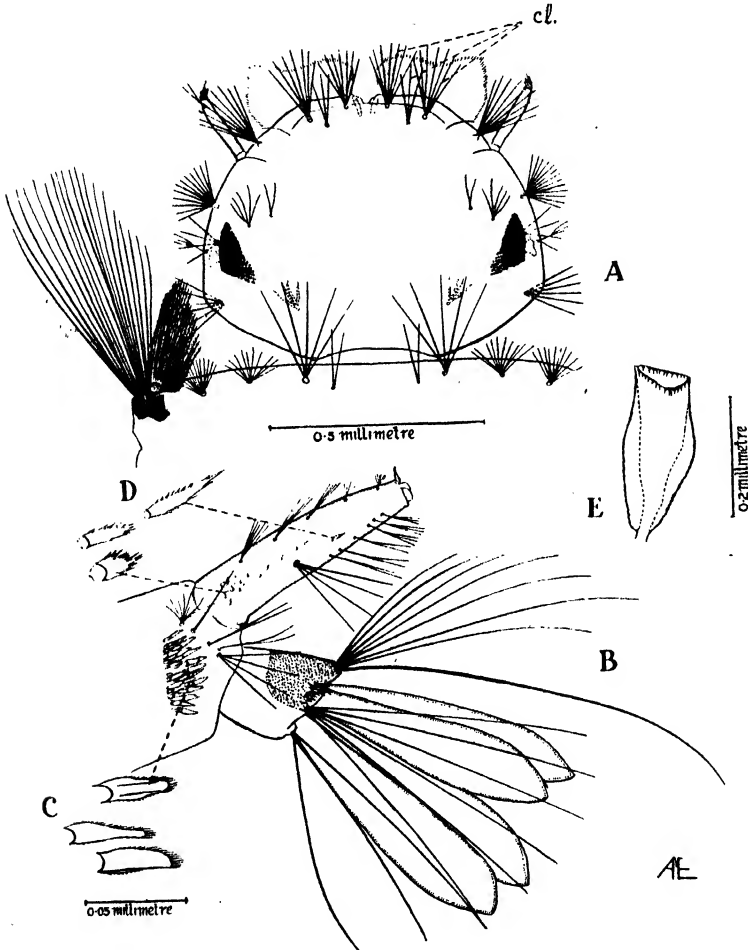


FIG. 1. *Harpagomyia farquarsoni* Edw. A.-D.—Fourth stage larva. A.—Head, dorsal aspect and anterior edge of prothorax (feathering of hairs not shown); B.—Terminal segments of larva, lateral aspect; C.—Spines of lateral comb; D.—Spines of pecten; E.—Pupal trumpet. A and B to same scale, C and D to same scale. cl.—Clypeal hairs.

Thorax. As in *H. trichorostris*, the prothorax bears dorso-laterally conspicuous, fan-like tufts of many feathered hairs arising from chitinous tubercles. There are a long and a short tuft on

each side in this species and a group of 3 long bristles completes the anterior dorso-lateral series ; the ventro-laterals are a single and a triple bristle and a shorter tuft. The hairs of the anterior border are shown in fig. 1, A. The meso- and metathorax bear fan-like tufts of hairs dorso-laterally, which are much less conspicuous than those of the prothorax. The dorso-lateral series of the mesothorax is completed by two long, simple, and one double bristle, and a much shorter, fan-shaped tuft. The ventro-laterals are a long fan-shaped tuft and a single bristle. The mesothorax also possesses a dorsal row of 4 simple and a bifid hair at each side. The dorso-lateral series of the metathorax are a long and a short fan-shaped tuft and there is a well-developed tuft ventro-laterally. Dorsally, at each side, is a group of 4 short multiple hairs. There is no palmate bristle on this segment as in *H. trichorostris*.

Abdomen. On the first and second segments the dorso-lateral plume is composed of 6 to 8 long, forwardly-projecting, bent, plumose hairs and below them a 2- or 3-branched, shorter, stout, bent hair. On the first segment shorter tufts are arranged as follows : dorsally a quadruple tuft near the posterior border ; dorso-laterally a tuft and a simple and a bifid hair above the base of the plumes ; laterally, in front of the plumes, a longer fan-like tuft of about 9 straight hairs ; ventro-laterally a tuft of 5 to 7 delicate hairs below the plumes, and more anteriorly and medially a tuft of 2 to 5 longer and rather stouter hairs. There is also a minute ventro-lateral tuft near the posterior border. The second segment only differs from the first in the possession of an additional minute, ventro-lateral tuft, and the absence of the anterior, ventro-lateral large tuft. On the remaining segments the dorso-lateral plumes consist of long, straight bristles, multiple (2 to 4), on the third to fifth segments, and single on the sixth and seventh. The dorsal and dorso-lateral tufts are similar in the last five segments to those of the first, but the posterior dorso-lateral tuft increases in size as far as the sixth segment ; on the seventh it appears to be absent, the dorsal tuft having become lateral in position. The large ventro-lateral series of tufts is well-developed and increases in size on the posterior segments, that of the seventh being longer than the segment. The ventro-lateral series of minute tufts and hairs increases in number posteriorly. The lateral comb consists of about 28 teeth and a few very small ones, arranged

in a sub-triangular patch, longer teeth as in fig. 1, C. The group of hairs and tufts beyond the comb is slightly variable, usually as shown in fig. 1, B. The siphon is rather weakly chitinised, the length about three and a half times basal width; it bears dorsally, two rows of 5 tufts, usually as in fig. 1, B, but variable and sometimes asymmetrical. Ventrally there is a single row of about 6 simple or 2- or 3-branched hairs; on the basal third each side bears a prominent tuft of 3 to 4 feathered hairs. The pecten is very variable in the number, arrangement and shape of the teeth. There is usually a basal patch of fringed teeth, which are sometimes short and broad, and an irregular row of elongated, fringed teeth extending along the basal two-thirds of the siphon, as in fig. 1, B. The tergite of the tenth or anal segment bears well-developed spines distally, as in *H. trichorostris*. The clinging (dorso-apical) bristles are usually a tuft of seven and a single very long bristle at each side. The ventral fin is represented by a pair of long double or triple hairs; a plume of 6 hairs arises laterally from the distal edge of the tenth segment. The anal gills are very long and wide, more than twice the length of the last segment.

This larva differs from that of *H. trichorostris* and *H. genurostris* chiefly in the large number of branches composing the clypeal and other head tufts. From the former it also differs in not possessing palmate tufts of short bristles on the metathorax. The great variability of the pecten spines is a noticeable feature.

Pupa. This is essentially similar to those of *H. trichorostris* and *H. genurostris*, possessing the peculiar triangular paddles which are fringeless and without any apical bristles, the fan-like tufts at the postero-lateral angles of the seventh and eighth abdominal segments and the long double post-ocular bristle. As in *H. trichorostris* the dentritic bristles on the first abdominal segment are rather poorly developed and the sublateral bristles are very long on segments five and six.

The cephalothorax is sub-pyriform in shape. The respiratory trumpet is rather short and stumpy, the length is about two and a half times the greatest width. When the pupa is mounted laterally, the trumpets appear widest in the middle (fig. 1, E). The opening is almost at right-angles to the long axis; seen from above it is about twice as long as wide and appears constricted at about the

middle. In addition to the long, double, post-ocular bristle is a short double, inferior one. The antero-thoracic are a single, two bifid and a trifid hair ; the dorsal hair is simple and external to it is a triple hair (? supra-alar). As in *H. trichorostris* the mid postero-thoracic hair is simple and the outer double, but the internal may be either single or double. The longer bristles on the abdominal tergites are as in *H. trichorostris*, but there are also a number of small, associated, single and double hairs not referred to in the description of that species. Of these there are 3 on the second, fourth and fifth segments, 2 on the seventh and 1 on the third and sixth. The paddles have the mid-rib indistinct.

The long, double post-ocular bristle ; small, triangular, fringeless paddles and the fan-like tufts on the seventh and eighth segments are characters supporting the view put forward by Edwards (1922), that the genus *Harpagomyia* should be included in the *Sabethine* group.

FEMALE. In one mature pupa the spermathecae are well shown. They are almost globular in shape, the median much larger than the other two, its diameter being at least one and a half times that of either. The specimens did not show a scaled area between the eyes.

Described from several larvae and pupae well preserved in spirit, taken in leaf-whorls of the pineapple plant, near Yaba, Southern Nigeria, 1927, Mr. L. H. Dunn.

The identification of the adults was kindly confirmed by Mr. F. W. Edwards.

Mr. Dunn states that he found the mosquito breeding in abundance in the central leaf-whorls of the pineapple plants in the bush near Yaba. He suggests that the fan-like tufts on the prothorax ' may signify that the larvae are able to move about in the leaf sheaths in order to remain in the water.'

CULEX (CULICIOMYA) CINERELLUS Edw.

It is probable that the larva illustrated by Wesché (1910) and described as '*Pectinopalpus fuscus* Theo. ? (1)' is that of *C. cinerellus*. As, however, there are certain differences between Wesché's larva and those from which Mr. Dunn has bred adults of *C. cinerellus*, the

latter are briefly described below. No description of the pupa appears to have been published.

Larva (fig. 2, C). This larva closely resembles that of *C. macfiei* Edw. (Macfie and Ingram, 1923), in the characters of the head and the shape of the siphon tube. The siphon measures about 1.2 mm.

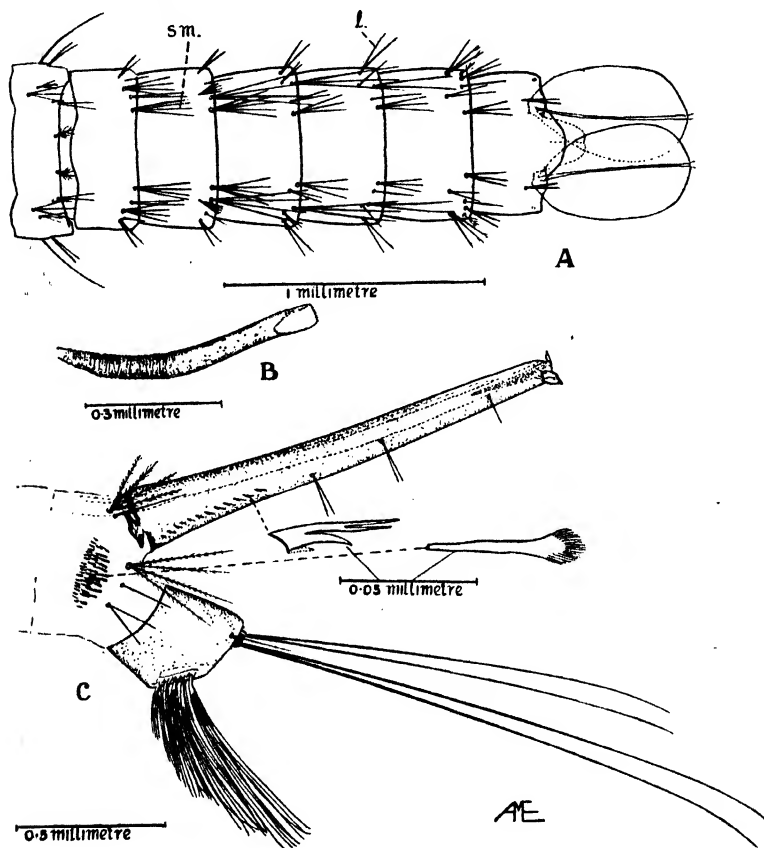


FIG. 2. *Culex* (*Culiciomyia*) *cinerellus*. A.—Abdomen of pupa, dorsal aspect, except first segment (very minute hairs not shown); B.—Pupal trumpet; C.—Terminal segments of fourth stage larva, spines of lateral comb and pecten shown enlarged as indicated; *l.*—Lateral bristle (Seta A); *sm.*—Submedian bristle (Seta C).

Head. The chaetotaxy and antennae are as in *C. macfiei*: the mental plate with 10 or 11 teeth on each side of the central tooth.

Thorax. The bristles of the anterior border are long; and lateral plumose tufts developed.

Abdomen. Lateral tufts are present on the first two segments. The lateral comb (fig. 2, C) consists of about 40 fringed spines, the posterior ones rather broad distally. Subsiphonal tuft of about 4 plumose hairs. The siphon is long and slender, the length about eight times the basal diameter; it is rather weakly chitinised. Two small double hairs and a distal simple hair lie on each side of the siphon; the arrangement of the proximal setae is variable. The pecten consists of about 12 spines which show, in a certain position, long bifid ends; each has a large tooth arising from the base and a very small basal tooth is frequently present. The tenth segment is lightly chitinised, and the ventral fin not well developed. The gills in the specimens are very long and thin, but the shape may have been altered in the process of moulting. They appear to be all of nearly equal length.

Pupa. The pupal pelt is small and lightly chitinised. The respiratory trumpet (fig. 2, B) is brown, darker on the basal half, and relatively narrow with a small opening. In one position the distal half is slightly wider than as shown in the illustration.

Abdomen (fig. 2, A). The paddles resemble those of *C. macfiei* in shape, but the terminal setae are very minute. The dorsal abdominal chaetotaxy differs considerably from that of *C. macfiei*, in the numbers of the branches of most of the tufts. In *C. cinerellus* the submedian ('Seta C,' Macfie, 1919), on segment three, has from 3 to 5 branches; that of the corresponding segment in *C. macfiei* being composed of 15 to 17 branches; on segments five, six and seven, these bristles are single in the latter species, but are triple in *C. cinerellus*. The laterals 'Seta A' on segments two to six are double in this species, but single in *C. macfiei*.

Described from several larval and pupal pelts of the specimens taken in crab-holes, Lagos, 1926, Mr. L. H. Dunn.

Mr. Dunn stated that he found this species breeding in abundance in many of the crab-holes in Lagos.

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NOTES ON CERTAIN VARIETIES OF *ANOPHELES MARSHALLI* THEOBALD

BY

A. M. EVANS

(Received for publication, 14 October, 1929)

During the course of his recent survey of the mosquitos of the Stanleyville Region of the Belgian Congo, Dr. J. Schwetz has bred out a number of specimens of *A. marshalli* var. *moucheti*, and a specimen which is evidently var. *hargreavesi*. From material which he has very kindly sent to me, I am able to describe the larva and pupa of the former and the pupa of the latter. As there are considerable differences between the pupa of *A. marshalli* var. *freetownensis* and both of those discovered by Dr. Schwetz, that of the Freetown variety is described as well. With the help of Dr. Schwetz's material it has been possible to examine the male terminalia of a series of var. *moucheti* and, as a constant distinction exists between that variety and var. *freetownensis* in these structures, I have also included a brief description of them and illustrated the chief points of difference.

ANOPHELES MARSHALLI var. *MOUCHETI* Evans

The larva and pupa of this variety differ rather markedly from those of *A. marshalli* var. *freetownensis*, and also from the larva of *A. marshalli* described by de Meillon, as well as from that of *marshalli* described by Macfie and Ingram.

Larva (figs. 1-3). A very small larva, this variety being of the same size as *funestus*.

Inner clypeal hairs long and simple; outer much shorter and branched, the number and arrangement of the branches somewhat variable (see fig. 1, *A*, *A'*). Posterior clypeal hairs minute and simple or with bifid ends; these are much shorter than those in var. *freetownensis* and are more anteriorly situated. Antenna with spiculated inner surface and minute hair towards the base; hair at apex between the two spines, dividing into three branches (apparently two in one specimen). Mental plate with the lateral teeth very uneven; those nearest to the apical tooth much smaller

than the next teeth (fig. 1, B). Anterior submedian thoracic hairs arising from a common but not well-chitinised base; the hairs are somewhat variable but show no marked difference from those of *A. marshalli* var. *freetownensis*. Metathorax with a pair of palmate bristles with narrow leaflets ending in long filamentous points as in var. *freetownensis*, leaflets about fourteen to sixteen in number.

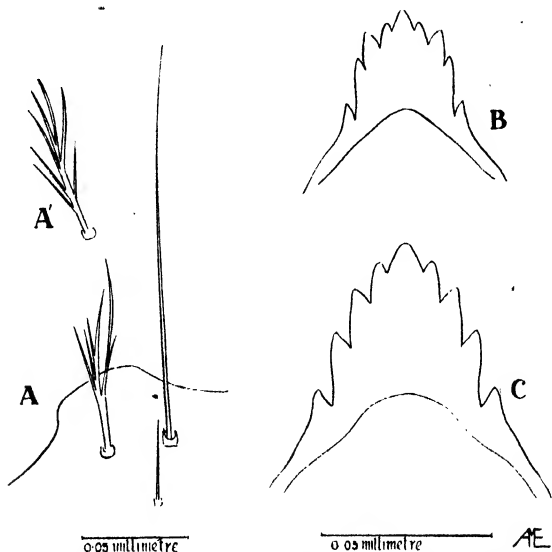


FIG. 1. A.—Clypeal hairs of larva of *A. marshalli*, var. *moucheti*; A'.—Outer clypeal hair of another specimen of same; B.—Mental plate of same; C.—Mental plate of larva of *A. marshalli*, var. *freetownensis*. B and C drawn to same scale. The essentials of these and the following figures were drawn with the aid of a camera lucida.

Abdominal tergal plates larger than those of var. *freetownensis* but not nearly so large as those of *funestus* (fig. 3). Palmate bristles present on abdominal segments one to seven, that of the first segment rudimentary, the leaflets not so well developed as in var. *freetownensis* (fig. 2, A, B). Palmate bristles on remaining segments slightly smaller than in var. *freetownensis*, the leaflets being slightly narrower and the filaments relatively much longer than in that variety (C, D). Lateral comb much as in var. *freetownensis*, the barbs on the teeth rather indistinct in the specimens.

This larva differs from those of *A. marshalli* from South Africa and its variety *freetownensis*, chiefly in the definitely branched outer clypeal hairs and in the relatively longer filaments of the abdominal

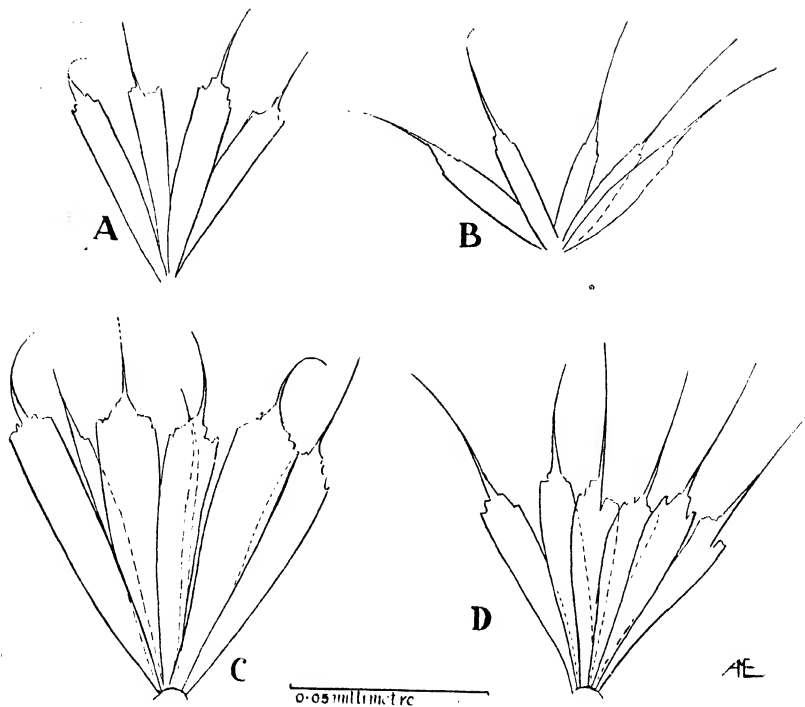


FIG. 2. *A*.—A few leaflets from median part of palmate bristle of first abdominal segment of larva of *A. marshalli*, var. *freetownensis*; *B*.—The same of var. *moucheti*; *C*.—The same of the fifth abdominal segment of var. *freetownensis*; *D*.—The same of var. *moucheti*.

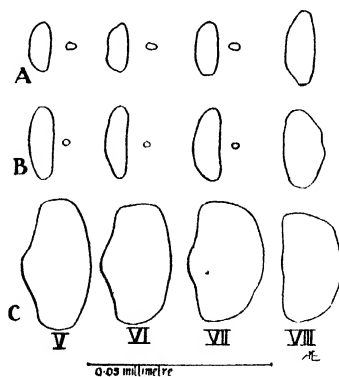


FIG. 3. Tergal plates of abdominal segments 5 to 8, as indicated. *A*.—*A. marshalli*, var. *freetownensis*; *B*.—var. *moucheti*; *C*.—*A. funestus*.

palmate bristles. It also differs from var. *freetownensis* in the very short posterior clypeal hairs and other characters noted above and illustrated in figs. 1-3, and from typical *marshalli* in the finely-pointed leaflets of the thoracic palmate bristles. The larva described as that of *A. marshalli*, by Ingram and Macfie, from the Gold Coast, differs very greatly from the three above-mentioned larvae in having no rudimentary palmate bristles on the thorax or first two abdominal segments, and the palmate bristles on the other segments are composed of leaflets which 'show no shoulder and scarcely any filament.'

Pupa (figs. 4, 5). The pupa is about equal in size to that of *funestus*.

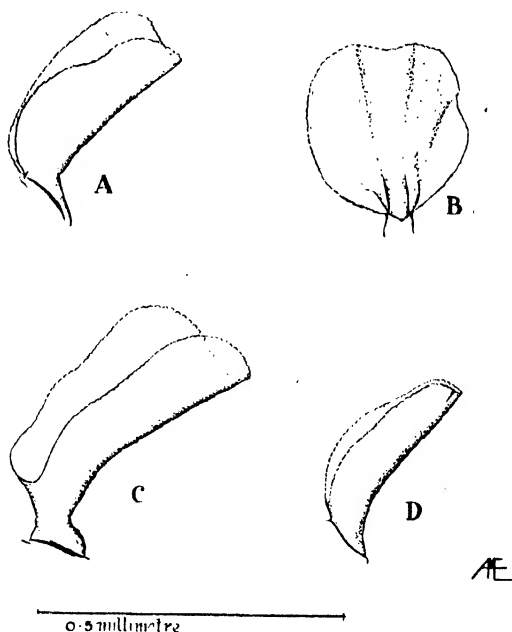


FIG. 4. Pupal respiratory trumpets. A. and B.—*A. marshalli*, var. *moucheti*, showing two typical aspects; C.—var. *freetownensis*; D.—var. *bargreavesi*.

The respiratory trumpets (fig. 4, A, B) are open to the base at one point, so that, although there is a small basal continuation, below the level of the opening, there is no true 'meatus' or tubular portion. Probably in consequence of this, the trumpets are often flattened out when mounted, as shown in fig. 4, B.

Abdominal chaetotaxy (fig. 5, A). The lateral bristles (*l.*), on segments four to seven, of the usual spine-like form, very short on the third segment and reduced to a minute tubercle on the second; on the eighth segment these bristles are fringed as usual, and are about one-fifth to one-sixth the length of the paddles. The submedian series (*sm.*), well developed and branched on the second

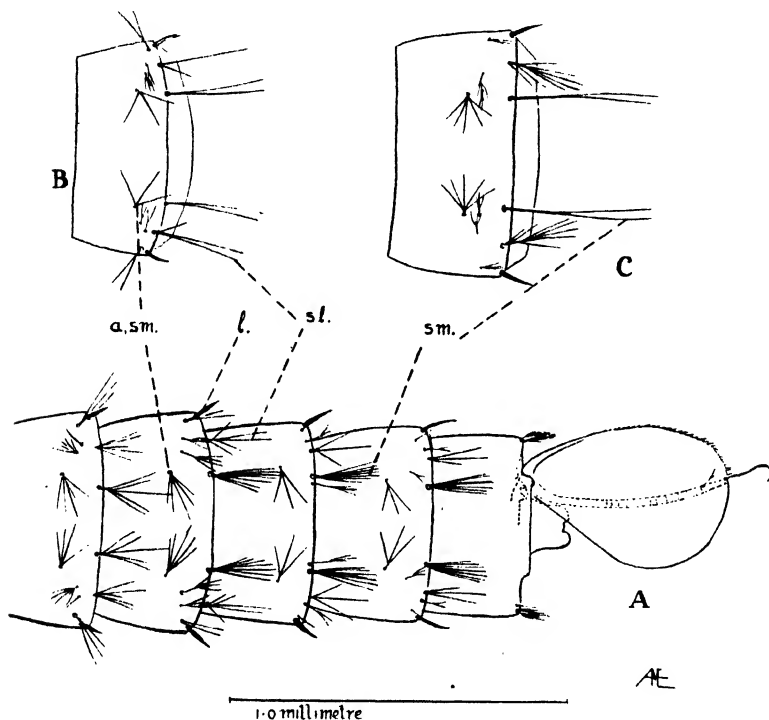


FIG. 5. A.—Abdomen of pupa of *A. marshalli*, var. *moucheti*, except first three segments, dorsal aspect; B.—Fifth abdominal segment of pupa of var. *bargreavesi*; C.—The same of var. *freetownensis*; a.sm.—anterior submedian bristle; *l.*—lateral; *sl.*—sublateral; *sm.*—submedian.

to the fourth segments, but as usual, more strongly developed on segments five to seven, where they form a strong tuft of 6 to 8 branches arising from a short stem. Sublateral series (*sl.*), well developed and branched on segments three to seven, consisting of 3 to 5 branches on the last four of these. Anterior submedian branched hairs (*a.sm.*), well developed and consisting of 3 to 5 branches on the fourth to seventh segments. Paddles of the type

broadly expanded distally, with fringe only on the outer side of the apical bristle, with well-developed ' buttress ' along the basal part of the outer side and apical bristle of the angularly hooked shape ; sub-apical hair very slender, usually 3-branched, the stem about equal in length to the branches. Fringe short and spine-like on the lateral margin, longer and hair-like on the distal margin, the change from one type to the other abrupt.

Described from one larval pelt and two larvae and numerous pupal pelts, taken from a pool (' Etang de la Mission, rive herbeuse ') ; and streams (' ruisseaux, herbes à sol '), August and September, 1928, Dr. J. Schwetz. Adults of typical *A. marshalli* var. *moucheti* were bred from the pelts.

Variation in female palpal markings. In two of the ♀♀ bred out by Dr. Schwetz, the palpi were entirely without the subapical dark ring, the whole of the outer third of the palpi being white-scaled.

Male terminalia. The claspers have the usual five parabasal spines which are similar in character to those of *funestus*, as illustrated by Christophers (1925). The lobes at the bases of the side pieces (harpagones, Christophers, 1915) with the apical bristle longer than the club and a shorter bristle externally. Phallosome or mesosome (fig. 6, B), with four or five leaflets at each side of the tip. Longest pair of leaflets much longer than those next to it and slightly serrated at the base.

Described from seven specimens, four of which were stained with carbol fuchsin ; all were mounted so as to display the mesosome.

***ANOPHELES MARSHALLI* var. *HARGREAVESI* Evans**

Amongst the material sent by Dr. Schwetz was a perfectly preserved female, with its pupal pelt, of a variety of *marshalli* which agreed extremely closely with that of var. *hargreavesi* from Sierra Leone. In the banding of the palps and tarsi and shape and size of the wing scales, the Congo specimen is indistinguishable from that variety. The third and fourth dark costal spots are somewhat greater in extent than in the type of *hargreavesi*, but in this they agree with a specimen of *hargreavesi* from Southern Nigeria. The thoracic

scales are of the broad type, much broader than in any other variety of *marshalli*, but not quite so broad or with such a large proportion of truncate scales as in the type of *hargreavesi*. From a study of four other West African specimens, three of which are Nigerian, there is little doubt, however, that Dr. Schwetz's specimen is within the limits of variation of that variety.

It should be noted here that the wing scales of var. *hargreavesi* are indistinguishable in size and shape from those of var. *moucheti*, and that the presence of a pale interruption in the third dark area on the first vein (R_1), appear to be a constant character.

Pupa (figs. 4, 5). This is a small pupa about equal in size to that of var. *moucheti*.

The respiratory trumpets appear to have a very short meatus, as shown in fig. 4, *D*, but the wall at the base of the opening is so thin that it is difficult to be certain how far the opening extends. The abdominal chaetotaxy differs from that of both var. *moucheti* and *freetownensis*, but the lateral series are very similar to those of the former. The submedian series are 3-branched on the second segment and longer and 3- or 4-branched on the third; on the fourth segment they are almost as well developed as on the fifth, and are a 2-branched bristle on one side, a 3-branched on the other. On the fifth to the seventh segments these are long, double bristles, as long as the tergites. The sublateral series on the fourth to sixth segments are long as in var. *moucheti*, but do not show more than three branches. The anterior submedian hairs have also fewer branches than in var. *moucheti*, having 3 branches where, in that variety, there are 5. The paddles have the distal margin appearing much less rounded internally than in var. *moucheti*; the fringe resembles that of *moucheti* in that there is a very sharp demarcation between the spine-like processes and the more distal hair-like processes. The apical bristles are not complete; the subapical hair is bifid or trifid, the stem being about equal to the branches, thus resembling that of var. *moucheti*.

Described from the pupal pelt of a specimen taken at the side of a stream, 'herbes à sol,' Simi-Simi, near Stanleyville, Belgian Congo, 30 August, 1928, Dr. Schwetz.

ANOPHELES MARSHALLI var. **FREETOWNENSIS** Evans

Pupa. This pupa is rather larger than that of var. *moucheti* (fig. 4, A and C, fig. 5, A and C). The respiratory trumpet closely resembles that of *funestus* in structure and differs from that of var. *moucheti* in that the opening is not continued to the base and thus a short tubular 'meatus' is present, which measures about one-fifth of the total length of the trumpet.

The abdominal chaetotaxy (fig. 5, C) differs markedly from that of var. *moucheti*, but bears a good deal of resemblance to that of *funestus*. Lateral series of spine-like bristles longer and rather more slender on the fifth to seventh segments than in var. *moucheti*: on eighth segment about as in var. *moucheti*. The submedian series on the second to fourth segments are branched hairs; on the fifth to seventh segments these are long bristles, either single or with 2 or, occasionally, 3 branches. Sublateral series on segments five to seven with 5 to 8 branches. Paddles differing from those of var. *moucheti* chiefly as follows: Subapical hair of 4 to 7 branches arising from a very short stem; spine-like part of fringe only extending a short distance and merging gradually into the fine hair-like part; fine processes of fringe longer than those in var. *moucheti*. Apical bristles bent into a hook as in var. *moucheti*.

Described from the pupal pelts of eight specimens of this variety collected in and around Freetown, Sierra Leone, 1925, by Professor Blacklock and the writer.

Male terminalia. Five parabasal spines similar to those of var. *moucheti* are usually present, but in one specimen there is an additional short, bent, internal spine, evidently an individual peculiarity. The lobes at the bases of the side-pieces usually have, in addition to the bristles present in var. *moucheti*, a short bristle just internal to the long one. Phallosome (fig. 6, A) with at least six pairs of leaflets, usually eight and sometimes nine or ten. The leaflets of each side are arranged in two or three series, the longer ones broad and serrated and sometimes with a median thickening, the length of the different leaflets more evenly graded than in var. *moucheti*.

Described from seven specimens, four of which were stained with carbol fuchsin and all mounted so as to display the mesosome.

In 1927 I suggested that a study of their early stages might throw light on the question of the status of the varieties of *A. marshalli*. The larvae of the type form from South Africa and three others are now known, and are all distinct from each other, while of the pupae of the three varieties here described, those of *moucheti* and *freetownensis* are easily separable from each other and from that of the form described by Ingram and Macfie (1917) from West Africa. The pupa here described as that of *hargreavesi*

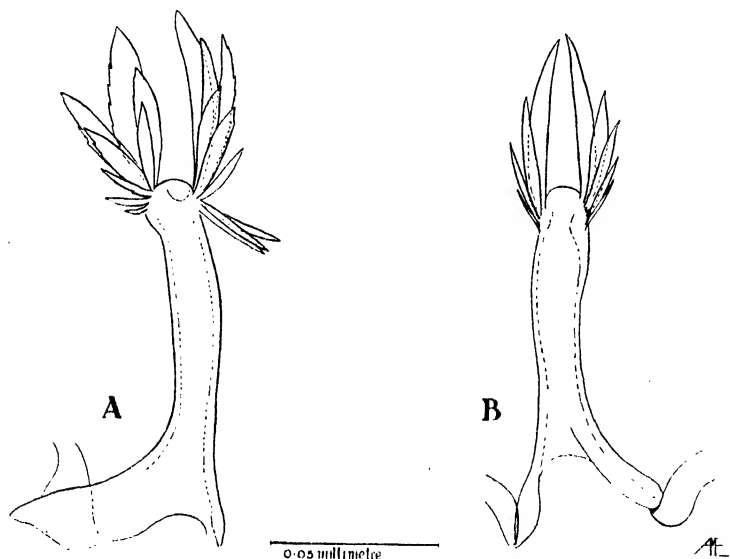


FIG. 6. A.—Phallosome of ♂ *A. marshalli*, var. *freetownensis*; B.—The same of var. *moucheti*.

is quite distinct from the former, and certainly also appears to differ from the latter. Another larva which may possibly be that of a variety of *marshalli*, was collected by the writer in a roadside ditch in the hills behind Freetown, Sierra Leone, in 1925. In addition to this larva, only a pupa was found in this ditch and it gave rise to a female which agreed very closely with *A. marshalli* var. *domicolus* Edw. The associated larva is quite different from those of any of the forms of *marshalli* so far described and appears to differ from that of any known African Anopheline larva. The palmate bristles are extremely small, slightly smaller than those of *costalis* and only about half the diameter of those of var. *freetownensis*.

The leaflets are relatively much wider than in *costalis* and the filaments much shorter, the proportions being about as in *freetownensis*. Rudimentary palmate bristles are present on the first two abdominal segments, but could not be detected on the thorax which is, however, distorted owing to the fact that the pupa was beginning to emerge. The clypeal hairs are all simple, the outer being about two-thirds the length of the inner, and arising some distance behind the bases of the latter. The lateral comb is rather similar to that of *freetownensis*, but the five short teeth between the lowest long teeth show a regular decrease in size from below upwards. The pupal respiratory trumpet, which is completely developed, has a short meatus and resembles that of *freetownensis*.

If this larva should prove to be that of *domicolus*, then that variety would be very distinct in its larval stage from the other forms of *marshalli* of which the larvae are known. It still remains to be seen whether the early stages of *pitchfordi*, *flavicosta*, and *austeni* are equally distinct. A good deal of confusion has existed regarding the diagnosis of the first of these. If, however, the larvae and pupae of specimens agreeing with the type series from South Africa, in having the thoracic scales almost hair-like on the posterior two-thirds, could be compared with those of *moucheti*, *hargreavesi* and *freetownensis*, it would probably be possible to define more clearly the characters of this variety. The differences which are shown to exist between the male terminalia of the two varieties, *moucheti* and *freetownensis*, suggest that a study of these structures in other varieties might afford another clue to the limits of variation and relationships of the forms of *marshalli*. It seems possible that one or more new forms may have to be regarded as distinct, or that some of the existing varieties be raised or re-raised to specific rank. It is obvious, however, that a thorough examination of the early stages and male terminalia of series of specimens showing many shades of variation will have to be made before a definite conclusion can be reached.

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The Manáos Research Laboratory

<i>Director</i>	. . .	HAROLD WOLFERSTAN THOMAS, M.D., C.M.
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Sierra Leone Research Laboratory

<i>Director</i>	. . .	Vacant.
<i>Assistant Director</i>	. . .	RUPERT MONTGOMERY GORDON, M.D.
<i>Research Assistants</i>	. . .	MARION WATSON, M.B. E. P. HICKS, M.B. T. H. DAVEY, M.B.

THE MARY KINGSLEY MEDAL

This medal was struck in commemoration of the work of the late Miss Mary Kingsley in West Africa, and is conferred in recognition of distinguished scientific achievement.

HONORARY RECIPIENTS

Her Royal Highness Princess Christian
Lord Lister
The Right Hon. Joseph Chamberlain
Prince Auguste d'Arenberg

Mrs. Pinnock
Mr. William Adamson
Professor William Carter

RECIPIENTS

1905—

Colonel Sir David Bruce, K.C.B.
Geheimrath Professor Robert Koch
Dr. A. Laveran
Sir Patrick Manson, K.C.M.G.

1907—

Professor Danielewsky
Dr. Charles Finlay
Mr. W. M. Haffkine
Professor Golgi
Colonel Gorgas
Professor Theobald Smith

1910—

Sir William Macgregor, G.C.M.G.
Professor R. Blanchard
Dr. Anton Breinl
Professor Angelo Celli
Dr. C. W. Daniels
Surgeon-General Sir Alfred Keogh
Colonel W. G. King
Professor Nocht
Professor G. H. F. Nuttall
Major Leonard Rogers
Professor J. L. Todd
Surgeon-General Walter Wyman

1913—

Professor Fred. V. Theobald

1917—

Dr. Griffith Evans

1919—

Dr. J. W. Scott Macfie
The Oswaldo Cruz Institute, Rio de Janeiro

1920—

Major E. E. Austen, D.S.O.
Dr. A. G. Bagshawe, C.M.G.
Dr. Andrew Balfour, C.B.
Dr. A. L. G. Broden
Mrs. Chalmers, in recognition of the
work of the late Dr. A. J. Chalmers
Professor B. Grassi
Professor R. T. Leiper
Professor F. Mesnil
Dr. Edmond Sergent
Dr. C. W. Stiles
Dr. T. Zammit

1929—

Dr. G. Carmichael Low
Dr. G. A. K. Marshall, C.M.G.
Professor R. Newstead
Dr. A. T. Stanton, C.M.G.
Professor J. W. W. Stephens
Dr. C. M. Wenyon, C.M.G., C.B.E.

THE ALAN H. MILNE MEDAL

This medal was struck to commemorate the late Alan H. Milne, C.M.G., the first Honorary Secretary of the School (1899-1917), and is awarded twice yearly on the recommendation of the examiners for the Diploma in Tropical Medicine.

1921—

George Phillip Farmer Allen

1922—

Quintin Stewart

1923—

John Cecil Cruickshank

1924—

George Maclean
Frederick John Carlyle Johnstone
Bernard Langridge Davis

1925—

Khwaja Samad Shah
Alfred Robert Davies Adams
Alfred J. Hawe

1926—

John McPhail Campbell
Triloki Nath Varma

1927—

Alexander M. Gillespie
Joseph Hector Pottinger
Ragade Sanjiva Rao

1928—

Joseph Fine

1929—

Robert Erskine Anderson
Aubrey Vernon Greaves
Ian Cameron Middleton

NOTICE

The following courses of instruction are given by the Liverpool School of Tropical Medicine each year :—

- (1) Two courses for the Diploma in Tropical Medicine, commencing on the 1st October and the 6th January. The D.T.M. examinations are held in December and March.
- (2) Two courses for the Diploma in Tropical Hygiene, commencing on the 13th January and the 23rd April. The D.T.H. examinations are held in March and July.
- (3) Two courses in Veterinary Parasitology, commencing on 1st October and the 7th January.

DIPLOMA IN TROPICAL MEDICINE

This Diploma shall be awarded only to candidates who possess a qualification to practise Medicine recognised for this purpose by the University, and who present satisfactory certificates of having attended approved courses of study, and pass the prescribed examination.

DIPLOMA IN TROPICAL HYGIENE

This Diploma can only be taken by those who have already obtained the D.T.M. of the University of Liverpool.

‘ The course for this Diploma will not be conducted unless at least five applications are received, and no application for admission can be considered later than December 21st and March 31st respectively.’

FEEES

D.T.M. Course	Twenty Guineas
D.T.H. Course	Ten Guineas
Course in Veterinary Parasitology				...	Fifteen Guineas
Each Diploma Examination			Five Guineas

Fee for use of a School microscope during one term ... One Guinea.

For prospectus and further information, application should be made to the Hon. Dean, School of Tropical Medicine, University of Liverpool.

The following have obtained the Diploma in Tropical Medicine of the University of Liverpool :—

Diploma in Tropical Medicine

<i>Date of Diploma</i>		<i>Date of Diploma</i>	
1904	Augustine, Henry Joshua	1907	Collinson, Walter Julius
1904	Bennett, Arthur King	1907	Davey, John Bernard
1904	Bruce, William James	1907	Donaldson, Anson Scott
1904	Byrne, John Scott	1907	Fell, Matthew Henry Gregson
1904	Clayton, Thomas Morrison	1907	Gann, Thomas William Francis
1904	Dalziel, John McEwen	1907	Graham, James Drummond
1904	Dee, Peter	1907	Iliscock, Robert Carroll
1904	Greenidge, Oliver Campbell	1907	Keane, Joseph Gerald
1904	Hehir, Patrick	1907	Kennan, Richard Henry
1904	Khan, Saiduzzafar	1907	Kenrick, William Hamilton
1904	Laurie, Robert	1907	Le Fanu, George Ernest Hugh
1904	MacLurkin, Alfred Robert	1907	Mackey, Charles
1904	McConnell, Robert Ernest	1907	Maddox, Ralph Henry
1904	Nicholson, James Edward	1907	McCarthy, John McDonald
1904	Philipson, Nicholas	1907	Raikes, Cuthbert Taunton
1904	Sharman, Eric Harding	1907	Ryan, Joseph Charles
1904	Thomson, Frank Wyville	1907	Vallance, Hugh
1904	Walker, George Francis Clegg		
1905	Anderson, Catherine Elmslie	1908	Caverhill, Austin Mack
1905	Brown, Alexander	1908	Crawford, Gilbert Stewart
1905	Caldwell, Thomas Cathcart	1908	Dalal, Kaikhusroo Rustomji
1905	Critien, Attilio	1908	Dansey-Browning, George
1905	Hooton, Alfred	1908	Davidson, James
1905	Hudson, Charles Tilson	1908	Dickson, John Rhodes
1905	Illington, Edmund Moritz	1908	Dowdall, Arthur Melville
1905	Macfarlane, Robert Maxwell	1908	Glover, Henry Joseph
1905	Maddock, Edward Cecil Gordon	1908	Greaves, Francis Wood
1905	Moore, James Jackson	1908	Goodbody, Cecil Maurice
1905	Nightingale, Samuel Shore	1908	Harrison, James Herbert Hugh
1905	Radcliffe, Percy Alexander Hurst	1908	Joshi, Lemuel Lucas
1905	Young, John Cameron	1908	Le Fanu, Cecil Vivian
		1908	Luethgen, Carl Wilhelm Ludwig
1906	Adie, Joseph Rosamond	1908	Mama, Jamshed Byramji
1906	Arnold, Frank Arthur	1908	McCay, Frederick William
1906	Bate, John Brabant	1908	McLellan, Samuel Wilson
1906	Bennetts, Harold Graves	1908	Pearce, Charles Ross
1906	Carter, Robert Markham	1908	Schoorel, Alexander Frederik
1906	Chisholm, James Alexander	1908	Smith, John Macgregor
1906	Clements, Robert William	1908	Stewart, George Edward
1906	Dundas, James	1908	Tate, Gerald William
1906	Faichnie, Norman	1908	Whyte, Robert
1906	Jeffreys, Herbert Castelman		
1906	Mackenzie, Donald Francis	1909	Abercrombie, Rudolph George
1906	Pailthorpe, Mary Elizabeth	1909	Allin, John Richard Percy
1906	Palmer, Harold Thornbury	1909	Armstrong, Edward Randolph
1906	Pearse, Albert	1909	Barrow, Harold Percy Waller
1906	Sampey, Alexander William	1909	Beatty, Guy
1906	Smithson, Arthur Ernest	1909	Carr-White, Percy
1906	Taylor, Joseph van Someren	1909	Chevallier, Claude Lionel
1906	Taylor, William Irwin	1909	Clark, William Scott
1906	Tynan, Edward Joseph	1909	Cope, Ricardo
1906	Watson, Cecil Francis	1909	Fleming, William
1906	Willcocks, Roger Durant	1909	Hanschell, Hother McCormick
1906	Williamson, George Alexander	1909	Hayward, William Davey
		1909	Henry, Sydney Alexander
1907	Allan, Alexander Smith	1909	Innes, Francis Alexander
1907	Allwood, James Aldred	1909	Jackson, Arthur Frame
1907	Bond, Ashton	1909	Kaka, Sorabji Manekji
1907	Branch, Stanley	1909	McCabe-Dallas, Alfred Alexander Donald

*Date of
Diploma*

1909 Meldrum, William Percy
 1909 Murphy, John Cullinan
 1909 Samuel, Mysore Gnananandaraja
 1909 Shroff, Kawsajee Byramjee
 1909 Thornely, Michael Harris
 1909 Turkhud, Violet Ackroyd
 1909 Webb, William Spinks
 1909 Yen, Fu-Chun

 1910 Brabazon, Edward
 1910 Castellino, Louis
 1910 Caulerick, James Akilade
 1910 Dowden, Richard
 1910 Haigh, William Edwin
 1910 Hamilton, Henry Fleming
 1910 Hefferman, William St. Michael
 1910 Hipwell, Abraham
 1910 Homer, Jonathan
 1910 Houston, William Mitchell
 1910 James, William Robert Wallace
 1910 Johnstone, David Patrick
 1910 Korke, Vishnu Tatyaji
 1910 Macdonald, Angus Graham
 1910 Macfie, John Wm. Scott
 1910 Manuk, Mack Walter
 1910 Murison, Cecil Charles
 1910 Nanavati, Kishavlal Balabha
 1910 Nauss, Ralph Welty
 1910 Oakley, Philip Douglas
 1910 Pratt, Ishmael Charles
 1910 Sabastian, Thiruchelvam
 1910 Shaw, Hugh Thomas
 1910 Sieger, Edward Louis
 1910 Sousa, Pascal John de
 1910 Souza, Antonio Bernardo de
 1910 Waterhouse, John Howard
 1910 White, Maurice Forbes

 1911 Blacklock, Donald Breadalbane
 1911 Brown, Frederick Forrest
 1911 Chand, Diwan Jai
 1911 Holmes, John Morgan
 1911 Ievers, Charles Langley
 1911 Iles, Charles Cochrane
 1911 Ingram, Alexander
 1911 Kirkwood, Thomas
 1911 Knowles, Benjamin
 1911 Liddle, George Marcus Berkeley
 1911 Lomas, Emanuel Kenworthy
 1911 Mackarell, William Wright
 1911 MacKnight, Dundas Simpson
 1911 Mascarenhas, Joseph Victor
 1911 Murray, Ronald Roderick
 1911 Oluwole, Akidiya Ladapo
 1911 Rao, Koka Ahobala
 1911 Sinton, John Alexander
 1911 Tarapurvala, Byramji Shavakshah
 1911 Taylor, John Archibald
 1911 Woods, William Medlicott

 1912 Aeria, Joseph Reginald
 1912 Anderson, Edmund Litchfield
 1912 Borle, James
 1912 Bowie, John Tait
 1912 Brassey, Laurence Percival ;

*Date of
Diploma*

1912 Christie, David
 1912 Dillon, Henry de Courcy
 1912 Dunn, Lillie Eleanor
 1912 Hardwicke, Charles
 1912 Jagose, Jamshed Rustumji
 1912 Kochhar, Mela Ram
 1912 McGusty, Victor William Tighe
 1912 Milne, Arthur James
 1912 Mitra, Manmatha Nath
 1912 Myles, Charles Duncan
 1912 Pelly, Huntly Nevins
 1912 Prasad, Bindeshwari
 1912 Prentice, George
 1912 Ross, Frank
 1912 Russell, Alexander James Hutchison
 1912 Ruthven, Morton Wood
 1912 Sandilands, John
 1912 Seddon, Harold
 1912 Smalley, James
 1912 Strickland, Percy Charles Hutchison
 1912 Watson, William Russel

 1913 Austin, Charles Miller
 1913 Banker, Shiavux Sorabji
 1913 Becker, Johann Gerhardus
 1913 Carrasco, Milton
 1913 Clark, James McKillican
 1913 Forsyth, Charles
 1913 Grahame, Malcolm Claude Russell
 1913 Grieve, Kelburne King
 1913 Hargreaves, Alfred Ridley
 1913 Hepper, Evelyn Charles
 1913 Hiranand, Pandit
 1913 Jackson, Oswald Egbert
 1913 Khaw, Ignatius Oo Kek
 1913 MacKelve, Maxwell
 1913 MacKinnon, John MacPhail
 1913 Macmillan, Robert James Alan
 1913 Mouat-Biggs, Charles Edward Forbes
 1913 Noronha, John Carmel
 1913 O'Connor, Edward
 1913 Olubomi-Beckley, Emanuel
 1913 Pestonji, Ardeshir Behramshah
 1913 Puttanna, Dodballapur Sivappa
 1913 Reford, John Hope
 1913 Smith, Edward Arthur
 1913 Stewart, Samuel Dudley
 1913 Walker, Frederick Dearden
 1913 Wilbe, Ernest Edward
 1913 Wilson, Hubert Francis
 1913 Yin, Ulg Ba
 1913 Young, William Alexander

 1914 Arculli, Hassan el
 1914 Chohan, Noormahomed Kasembha
 1914 Connell, Harry Bertram
 1914 Gerrard, Herbert Shaw
 1914 Gimi, Hirji Dorabji
 1914 Gwynne, Joseph Robert
 1914 Hodgkinson, Samuel Paterson
 1914 Jackson, Arthur Ivan
 1914 Kaushash, Ram Chander
 1914 Kelsall, Charles
 1914 Luanco y Cuenca, Maximino
 1914 Misbah, Abdul-Ghani Naguib

*Date of
Diploma*

1914	Naidu, Bangalore Pasupulati Balakrishna
1914	Rowe, John Joseph Stephen
1914	Roy, Raghu Nath
1914	Shiveshwarkar, Ramchandra Vishnu
1914	Sur, Sachindra Nath
1914	Talati, Dadabhai Cursedji
1914	Wilkinson, Arthur Geden
1914	Wright, Ernest Jenner
1915	Lobo, John Francis
1915	Madhok, Gopal Dass
1915	Pearson, George Howorth
1915	Swami, Karumuri Virabhadra
1915	Wood, John
1916	Barseghian, Mesroob
1916	Chaliha, Lakshmi Prasad
1916	Lim, Albert Liat Juay
1916	Lim, Harold Liat Hin
1916	Metzger, George Nathaniel
1916	Söderström, Erik Daniel
1916	Wheeler, Louis
1917	Chapman, Herbert Owen
1917	Krishnamoorthy, Yedatore Venkoba
1917	Lipkin, Isaac Jacob
1918	Watts, Rattan Claud
1919	Bowle-Evans, Charles Harford
1919	Burnie, Robert McColli
1919	Celestin, Louis Abel
1919	Cummings, Eustace Henry Taylor
1919	Darling, Georgina Renington
1919	Drake, Joan Margaret Fraser
1919	Fraser, William James
1919	Gordon, Rupert Montgomery
1919	Krige, Christian Frederick
1919	Maplestone, Philip Alan
1919	Oluwole, Isaac Ladipo
1919	Rustomjee, Khushshuyee Jamesidjee
1919	Sawers, William Campbell
1919	Thompson, Mary Georgina
1919	Turner, Gladys Maude
1919	Young, Charles James
1920	Adler, Saul
1920	Anderson, William Jenkins Webb
1920	Campbell, George
1920	Cobb, Charles Eric
1920	Cobb, Enid Margaret Mary
1920	Connolly, Evelyn Mary
1920	Fernandez, Daniel David
1920	Lim, Chong Eang
1920	McHutcheson, George Browne
1920	van der Merwe, Frederick
1920	O'Farrell, Patrick Theodore Joseph
1920	Renner, Edowo Awunor
1920	Vaughan, James Churchwill
1920	Waller, Harold William Leslie
1921	Allen, George Phillip Farmer
1921	Corfield, Charles Russell
1921	Hamid, Abdul
1921	Longhurst, Bell Wilmott
1921	Macvae, George Anthony
1921	Madan, Hans Raj
1921	Mulligan, William Percival

*Date of
Diploma*

1921	Nixon, Robert
1921	Richmond, Arthur Stanley
1921	Shri Kent, Shamsher Singh
1921	Skinner, James Macgregor
1921	Stewart, Robert Bell
1921	Thomson, Marion
1922	Bhatia, Jagat Ram
1922	Cohen, Morris Joshua
1922	Crawford, Andrew Clemmey
1922	Gilmore, Edward Raymond
1922	Gracias, Cajetan Manuel
1922	Jennings, Arthur Richard
1922	Lethem, William Ashley
1922	Paul, Sachchidananda Hoshen
1922	Pinder, John
1922	Riele, Stanley Desmond
1922	Rutherford, Gladys
1922	Stewart, Quintin
1923	Abelman, B.
1923	Basu, Dharendra Nath
1923	Cruikshank, John Cecil
1923	Doherty, Winifred Irene
1923	Edghill, Winifred M.
1923	Elsohn, John
1923	Fraser, N. D.
1923	Lee, R.
1923	Pierce, E. R.
1923	Raja, Rojaporum
1923	Reid, C. B. B.
1923	Richmond, A. E.
1923	Steven, J. B.
1923	White, Charles Francis
1924	Bilimoria, H. S.
1924	Carson, J. C.
1924	Chopra, B. L.
1924	Davis, B. L.
1924	Hardy, M. J.
1924	Jennings, C. B.
1924	Johnstone, F. J. C.
1924	Keirans, J. J.
1924	Lee, S. W. T.
1924	Macdonald, G.
1924	Maclean, G.
1924	Mathur, W. C.
1924	Mitchell, J. M.
1924	Owen, D. Uvedale
1924	Palmer-Jones, Beryl
1924	Sankeralli, E. J.
1924	Singh, H.
1924	Theron, Elizabeth M.
1925	Adams, Alfred Robert Davies
1925	Ashton, Frank Richard
1925	Ashworth, Esther
1925	Bamford, Charles Walker
1925	Beinashowitz, Jack
1925	Black, John
1925	Clark, George
1925	Coghlan, Bernard A.
1925	Collier, Ivy
1925	Crawford, E. J.
1925	Cumming, Patrick Grant

*Date of
Diploma*

1925 Ellam, Mary Muriel
1925 Fisher, Morris
1925 Green, Frederick Norman
1925 Grutu, M. S.
1925 Hawe, Albert J.
1925 Jafri, Z. H.
1925 Johnstone, Elvy I.
1925 Kerr, James R.
1925 Mackay, Donald M.
1925 Mackay, E. K.
1925 Makkawi, M.
1925 Maldonado, Leopoldo Garcia
1925 Mar, Severo Francisco
1925 Mozoomdar, B. P.
1925 Shah, Khwaja Samad
1925 Skan, Douglas A.
1925 Stone, Ernest R.
1925 Terrell, C. G.
1925 Tooth, Frederick
1925 de Waal, Jacobus Johannes

1926 Aitken, W. J.
1926 Ashworth, A.
1926 Austin, T. A.
1926 Bansikar, R. N.
1926 Besson, W. W.
1926 Bligh-Peacock, R. N.
1926 Bolton, Effie G.
1926 Boodrie, E. H.
1926 Brito-Mutunayagam, M. A. B.
1926 Campbell, J. McP.
1926 Cullen, T.
1926 Davies, H. E.
1926 Dias, B. G. V.
1926 Doherty, H. A. A.
1926 Don, E. G.
1926 Earl, J. C. St. G.
1926 Fletcher, Beatrice N.
1926 Fowler, H. P.
1926 Fowler, Isabella J.
1926 Hamilton, J.
1926 Hodgkinson, Katharine M.
1926 Jackson, R.
1926 Kamakaka, K. H.
1926 Kennedy, J. H.
1926 Khatri, L. D.
1926 Lennox, D.
1926 Lewis, A. J.
1926 McConn, C. F.
1926 Mackay, A. G.
1926 McLean, N.
1926 MacSweeney, M.
1926 Malhautra, K. L.
1926 Malik, S. B.
1926 Manuwa, S. L. A.
1926 Merchant, M. E.
1926 Mitchell, W. H.
1926 Molony, E. F.
1926 Nashikkar, S. G.
1926 Oppenheimer, F.
1926 Ormiston, W. S.
1926 Paterson, F. S.
1926 Patterson, F. L.
1926 Pouri, V.
1926 Quigley, L. D.
1926 Robertson, A.

*Date of
Diploma*

1926 Rodrigues, N.
1926 Sachdev, A. S.
1926 Singh, B.
1926 Singh, J.
1926 Talib, S. A.
1926 Tan, C. L.
1926 Taylor, Catherine F.
1926 Turnbull, N. S.
1926 Turner, J. G. S.
1926 Vardya, B. K.
1926 Varma, T. N.
1926 Voigt, C.
1926 Wasti, S. N.

1927 Allen, C. P.
1927 Bahl, M. I.
1927 Barrowman, B.
1927 Bawa, H. S.
1927 Bilimoria, J. D.
1927 Burns, W. M.
1927 Daly, E. J.
1927 Dunlop, G. A.
1927 Dyream, V.
1927 Evans, R. R.
1927 Farid, M.
1927 Gillespie, A. M.
1927 Gunawardana, S. A.
1927 Harkness, J.
1927 Hay, R.
1927 Hodivala, N. M.
1927 Hughes, Emma
1927 Hyslop, Kathleen M.
1927 Ingram-Johnson, R. E.
1927 Kapadia, J. S.
1927 Khan, F. A.
1927 Khan, M. M.
1927 Labuschagne, P. N. H.
1927 Laird, W. J.
1927 Lewin, B. F.
1927 Macdonald, J.
1927 McElroy, R. S.
1927 Maclay, W. S.
1927 Maguire, H. G.
1927 Mahaffy, A. F.
1927 Malhotra, A. H.
1927 Malhotra, A. L.
1927 Manghirmalani, B. S.
1927 Meek, A. I.
1927 Mehra, J. N.
1927 Mehta, H. C.
1927 Menon, M. V.
1927 Miller, H. V. R.
1927 Mokand, S. N.
1927 Murgatroyd, F.
1927 Murray, A. J.
1927 Murray, Pauline V.
1927 Nevin, H. M.
1927 Nirula, P. N.
1927 Olusoga, N. T.
1927 Parakh, D. B.
1927 Peters, D. O.
1927 Peters, M. R.
1927 Pottinger, J. H.
1927 Rao, R. S.
1927 Rodriguez, G. V. S.
1927 Shah, S. R. A.

*Date of
Diploma*

1927	Singh, H.
1927	Southward, J. F.
1927	Sturton, S. D.
1927	Thompson, Frances C.
1927	de Villiers, B. J. van de S.
1927	Walkinshaw, R.
1927	Wilkinson, S. A.
1928	Ahluwalia, C. L.
1928	Aidin, A. R.
1928	Anand, J. S.
1928	Askari, S. W. H.
1928	Beveridge, Ruby S.
1928	Biswas, M. K.
1928	Blakemore, W. L.
1928	Campa-Campins, J. M.
1928	Chacko, M. O.
1928	Chopra, A. N.
1928	Chaudhuri, J. P.
1928	Choudari, K. V. R.
1928	Cranage, Margaret
1928	Dhala, C. H.
1928	Dhar, K. K.
1928	Dikshity, H. K.
1928	Everard, N. J.
1928	Fine, J.
1928	Ghei, A. N.
1928	Halawani, A.
1928	Henshaw, L. E. R.
1928	Hilmy, I. S.
1928	Holmes, W. E.
1928	Hope-Gill, C. W.
1928	Kane, F.
1928	Katial, C. L.
1928	Khan, F. M.
1928	Krishna, R.
1928	Lawrence, H. S.
1928	Lawrence, M. R.
1928	McLaren, D. W.
1928	Malhotra, B. D.
1928	Mallick, B. D.
1928	Mason, Jean R.
1928	Menon, E. S. R.
1928	Milne, J.

*Date of
Diploma*

1928	Mitchell, A.
1928	Mone, R. V.
1928	Morley, A. H.
1928	Mostert, H. van R.
1928	Muffy, S.
1928	van Niekerk, S. V.
1928	Pandit, M. K.
1928	Pearce, W. T. A.
1928	Plum, D.
1928	Rao, B. D.
1928	Reid, A.
1928	Sanderson, I.
1928	Setna, H. M.
1928	Shearer, G.
1928	Singh, B.
1928	Sivalingam, S.
1928	Stratton, Ella M.
1928	Suri, R.
1928	Tuli, R. L.
1928	Udvardia, F. F.
1928	Wagle, P. M.
1928	Wahid, A.
1928	Wall-Mesham, Nellie
1928	Whig, P. L.
1929	Chakravarti, K. B.
1929	Crawford, J.
1929	Dale, W. C.
1929	Dogra, J. R.
1929	Drury, G. D.
1929	Gill, T. S.
1929	Herbertson, Margaret A. L.
1929	Innes, J. A. L.
1929	McGregor, J. A.
1929	McQueen, W. B.
1929	Majumdar, B. K.
1929	Middleton, I. C.
1929	Pearse, J. T. F.
1929	Ramdeholl, C.
1929	Robinson, Elizabeth J.
1929	Robinson, P. B.
1929	Shafi, A.
1929	Vergheze, T.
1929	Wilson, S. P.

The following have obtained the Diploma in Tropical Hygiene of the University of Liverpool:—

Diploma in Tropical Hygiene

*Date of
Diploma*

1926	Aitken, W. J.
1926	Bligh-Peacock, N.
1926	Clark, G.
1926	Collier, Ivy
1926	Cullen, T.
1926	Davis, B. L.
1926	Don, E. G. A.
1926	Fowler, H. P.
1926	Hawe, A. J.
1926	Lennox, D.
1926	Mackay, A. G.
1926	Mackay, D. M.
1926	McLean, N.

*Date of
Diploma*

1926	MacSweeney, M.
1926	Oppenheimer, F.
1926	Skan, D. A.
1926	Talib, S. A.
1926	Turnbull, N. S.
1927	Allen, C. P.
1927	Austin, T. A.
1927	Besson, W. W.
1927	Dunlop, G. A.
1927	Earl, J. C. St. G.
1927	Hamilton, J.
1927	Harkness, J.

*Date of
Diploma*

1927 Hay, R.
1927 Hyslop, Kathleen M.
1927 Labuschagne, P. N. H.
1927 McCon, C. F.
1927 Macdonald, J.
1927 Mitchell, Winifred H.
1927 Murray, A. J.
1927 Nevin, H. M.
1927 Nixon, R.
1927 Ormiston, W. S.
1927 Robertson, A.
1927 Walkingshaw, R.

1928 Bilimoria, J. D.
1928 Blakemore, W. I..
1928 Choudari, K. V. R.
1928 Dhar, K. K.
1928 Evans, R. R.
1928 Holmes, W. E.
1928 Laird, W. F.
1928 Maclay, W. S.
1928 Miller, H. V. R.
1928 Morley, A. H.
1928 Pearson, G. H.
1928 Pottinger, J. H.
1928 Sanderson, I.
1928 Sivalingam, S.
1928 Wilkinson, S. A.

*Date of
Diploma*

1929 Ahuja, S. D.
1929 Anderson, R. E.
1929 Askari, S. W. H.
1929 Booker, C. G.
1929 Bullen, W. A.
1929 Callum, E. N.
1929 Cole, H. A.
1929 Connolly, P. P. D.
1929 Cowan, J. A.
1929 Drury, G. D.
1929 Fraser, N. D.
1929 Graham-Cumming, G.
1929 Greaves, A. V.
1929 Halawani, A.
1929 Hale, G. S.
1929 Hilmy, I. S.
1929 Howell, A. T.
1929 Innes, J. A. L.
1929 Latham, C. N.
1929 Lawrence, H. S.
1929 McMahon, J. E.
1929 Miller, A. A.
1929 Ramdeholl, C.
1929 Rosenbloom, A.
1929 Row, C. K.
1929 Setna, H. M.
1929 Sewal, R. N.
1929 Singh, H.
1929 Talwrn-Jones, G. A.
1929 Turner, H. N.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY

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- ROBINSON, S. (1914). The spleen in malaria. *Ann. of Nosology*,
20, 20-25.
SMITH, J. (1900). Enlargement of the spleen in malaria. *Jl. of*
Pathometry, **1**, 1-20.

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THE EFFECTS OF SELECTION UPON SUSCEPTIBILITY TO BIRD MALARIA IN *CULEX PIPIENS* LINN.

BY

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(*Received for publication 1 August, 1929*)

PLATES VI AND VII.

INTRODUCTION

For one who is interested in the study of the biology of parasitism, there is no better material from which to choose than one of the malarial organisms. It has no free-living stages; it invades the tissues of both hosts; and it displays a specificity of attack which has been considered classic. The present investigations are the result of a desire to follow the purely biological questions involved in such a case of parasitism. Surely, there is no more interesting and important biological problem remaining unsolved than that of the specificity displayed by certain parasites in the selection of their hosts. Among such parasites the malarias are none the less interesting biologically because of their economic importance.

The present investigations have been concerned with one aspect of the problem of explaining the cause and nature of specificity. In a previous publication (1927) I brought forth evidence in favour of the existence of an *individual* immunity within a species of mosquito known to be susceptible to infection. The case cited (and the one with which we shall deal in this discussion) is that of the parasitism of *Culex pipiens* by *Plasmodium cathemerium* Hartman. As brought out previously (1927), this avian malaria is infectious only to certain individuals of *Culex pipiens*, in spite of the fact that every effort was made to see that each individual received thousands of gametocytes in its infective meal. If one feeds the mosquitos upon birds

* National Research Fellow. This research was supported by a grant from the Wellington Fund. I wish to express my gratitude to Dr. L. R. Cleveland for his interest and aid.

at the time when gametocytes are most numerous and, later, dissects the mosquitos, he will find that certain individuals have escaped infection, while the others have acquired tremendous infections. That the uninfected ones failed to acquire the infection because of a chance unequal distribution of parasites is an untenable hypothesis when the massive dose is taken into consideration. A part of Table VII is here reproduced from my previous paper (1927), to give specific illustration of the question at hand.

TABLE I.

Comparison of degree of infections in the birds, with the results of the dissection of mosquitos having fed from them. (Condensed from Table VII, p. 723, of 1927 publication.)

Lot	Parasites per 10,000 R.B.C.	Percentage gametocytes	Number gametocytes per 10,000 R.B.C.	Total Number dissected	Number positive
A	1,213	37.0	449	15	7
D	778	10.0	78	9	4
F	784	8.7	63	7	3
I	243	14.0	34	16	10
J	1,288	2.0	26	8	4

In that paper it was calculated (pp. 712-713) that most of these mosquitos received several hundreds or several thousands of gametocytes when they took their infective meals. It seems that here is some inherent ability to evade infection. The natural question then arose: Is this ability of certain individuals for resisting infection hereditary, or do environmental factors play the determining part?

SPECIES USED AND THE REASONS FOR THEIR CHOICE

This study was conducted upon *Plasmodium cathemerium*, with the domestic canary serving as vertebrate host and *Culex pipiens* as the invertebrate host. Of the three species of avian Plasmodiums available, *cathemerium** was most satisfactory for this study because

* The question of the nomenclature of this species will be treated in a subsequent publication. The parasite has been known in the United States as the 'Hartman strain' of bird malaria, and was designated *Plasmodium cathemerium* by Hartman (1927b). It is the species used by the following writers in the publications listed. Taliaferro (1925) called it the 'recently isolated strain,' Hegner and MacDougall (1926) did not give it a scientific designation, Hartman (1927a) called it *P. praecox*, and Boyd (1929) has published upon it under the name *cathemerium*.

of the higher infections produced by it in the bird. *Culex pipiens* was chosen as the insect host because of its preference for avian blood, of the ease of breeding it in captivity, and of the existence of an individual immunity already demonstrated.

METHODS EMPLOYED

The strains of mosquitos used were obtained by collecting the larvae of adults from the field. In the case of lines *A*, *B*, and *C*, to be described later, large quantities of larvae were collected from a stagnant swamp in August, and brought into the laboratory and bred out. Lines *D* and *E* were started from hibernating females caught in a cellar in February, by Dr. Marshall Hertig, to whom I wish to express my thanks at this point. Some difficulty is encountered by either of these methods in getting this species to breed through the first few generations. This is due to the fact that only a very few of the first generation will copulate in captivity. Many rafts of eggs will be laid but only rarely will they be viable. However, if these are carefully nursed through the next generation they will then usually copulate readily even in lamp chimneys and practically all of the eggs laid will hatch.

The technique for handling the mosquitos has previously been described (1927, pp. 712-715). The larvae were grown in large white bowls of water to which a small amount of dehydrated blood serum and milk powder was added daily. These bowls were kept covered to prevent chance ovipositions from extraneous females. When pupae appeared they were removed and placed in crystallizing dishes over which lantern globes with gauze coverings were fitted. The adults emerged into this globe cage and the latter was placed over a Petri dish containing a moist cotton pad. Feeding upon infected birds was accomplished by tying the immobilized bird upon the top of the cage in such a manner that the mosquitos could bite it in the pectoral region. After a lot of mosquitos had been fed upon a severely infected bird, the engorged ones were separated from the unengorged and the former properly labelled and put aside for five days. Then the females were each put into separate oviposition chambers containing water, and given serial numbers. As soon as ova were laid or the female died, the latter was dissected

and the stomach examined for the presence of oocysts. If the female was infected, her progeny was kept and the above process repeated upon it, again selecting the progeny from infected females. Such a line was called a 'Positive' or 'Susceptible' line. From the progeny of females showing no infection in the first generation, 'Negative' and 'Non-susceptible' lines were begun by the same method.

One of the most important points in this technique was the need for feeding all mosquitos on birds with the highest possible infections. It was especially important to make sure that the percentage of gametocytes was high as well as to select birds with infections in which the total numbers of parasites were high. In this work enough infected birds were kept on hand to provide the desired type of infection at the desired time. In dealing with the negative lines of mosquitos it was necessary to run control feedings from stock mosquitos in order to be sure that the bird upon which they were fed possessed highly infectious blood.

All mosquitos were kept in a constant temperature room which was equipped with thermostat and electric heater. The temperature range was 79°-82° F. (26.0°-27.7° C.). The relative humidity of the room was kept high by allowing the water to drop from the hot-water faucet. The moisture content of the breeding cages was usually higher than that of the room because of the use of a saturated pad of wood-fibre cotton on the bottom of each. Inasmuch as all lots of mosquitos were kept under identical conditions of temperature and moisture, it is believed that these environmental conditions could have played no differential part in the infections of the individuals of a given lot.

It seems desirable to record here some of the difficulties which one encounters in this type of experiment; for it is realised that the numbers of generations and the number of individuals in each generation dealt with fall far short of the numbers required by those interested purely in the genetics of the problem. First, there are usually only about fifty to two hundred ova laid by a single female. If all of these hatch and grow to become adults—which seldom happens—half of them will be males. Then, when the remainder are given an opportunity to feed upon an infected bird they often show great indifference to the opportunity and many of them will die of

starvation rather than suck blood. Of those fed, a large proportion survive long enough to allow oocysts to develop sufficiently to be seen upon dissection. However, not all of them lay eggs, and even some of the eggs laid are not viable. When it is remembered that some of these larvae from the viable ova will have to be discarded in the selection of a susceptible or non-susceptible line, it will be seen that the vicissitudes are many and the successes few. Genetical problems upon this line of work are ones to be undertaken by a staff of workers rather than by an individual. It is, of course, important to remember even in this case that one is dealing with a character which manifests itself only in the female and that the male, therefore, always remains an unknown factor.

The difficulties involved ought not to be blamed entirely upon the species of mosquito used. The fact is that for mass breeding *Culex pipiens* makes a splendid laboratory animal. That it is also a good species for certain types of genetical experimentation I hope to show in a subsequent publication, the data for which are now nearly completed.

TABULATION OF RESULTS

An attempt has been made here to make the results obtained as clear as possible by representing them in several different ways. Table II gives the dissection results for the various selected lines. After the explanation already made about the many difficulties in the way of carrying the selection for many generations, no apology is made for the brevity of some of the lines. It should, however, be said in justice to the technique used, that line *D* came to an end because all of the ova laid by the second generation were non-viable. Line *E* was saved only by using the progeny of an uninfected mother (from the only ova which hatched) instead of that from an infected mother as should have been done if the latter had been available. No males were obtained in the following generation; so males from *B-VI* were inserted, since they were the only ones available at the time.

Figs. 1 and 2 present the same results in graphic form which, it is hoped, will render a more lucid comparison of the two kinds of strains possible. Fig. 1 is constructed from the actual numbers

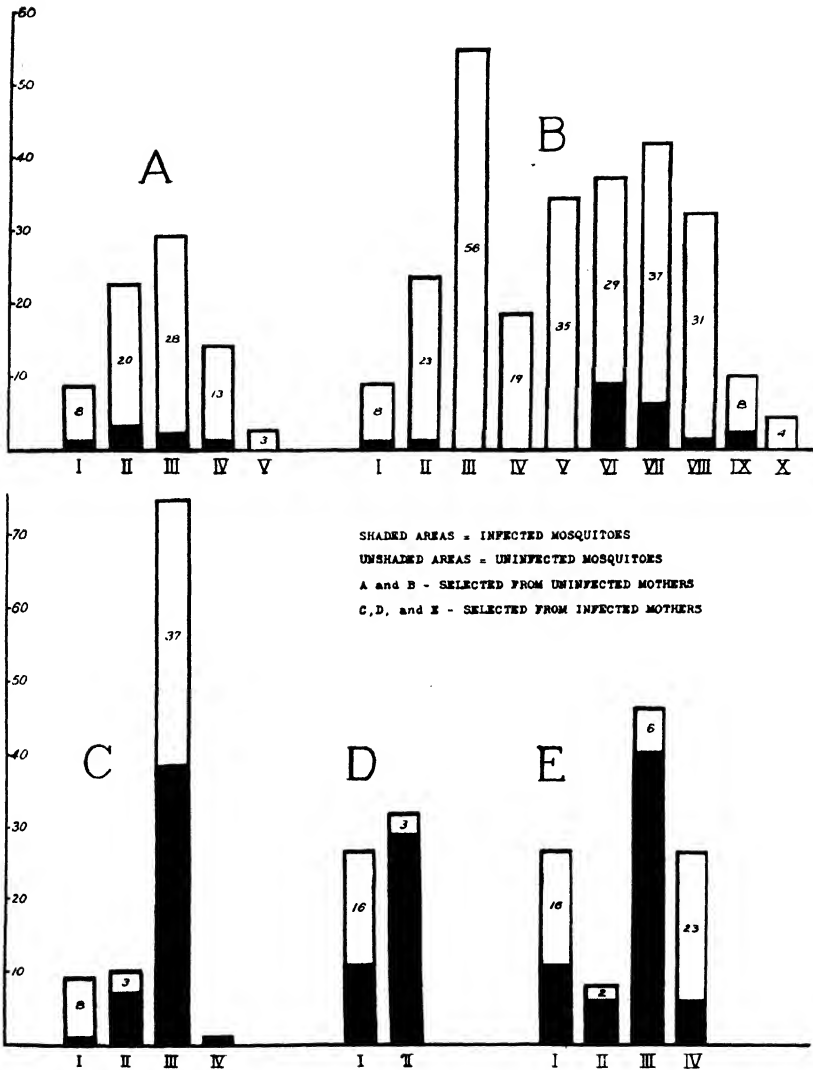


FIG. 1. Block Graph showing numbers of infected (shaded areas) individuals and uninfected (unshaded areas) individuals in each generation.

of positive and negative dissections, while fig. 2 shows percentages of the infected individuals in a given generation expressed in terms of the total number dissected. Plate VI is an attempt to represent graphically the pedigree of the strains. It has been possible here to represent more clearly the origins of the various strains and to indicate the kind of selection used. It will be seen from this figure

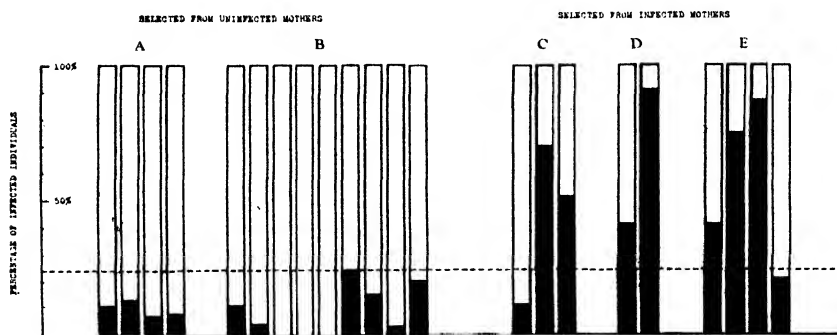


FIG. 2. Block Graph showing the percentages of infected individuals in a given generation expressed in terms of the total number dissected. The dotted line shows the percentage of individuals infected in unselected lots.

that strains *A*, *B*, and *C* came from the same original group of mosquitos (from the progeny of a single female); and also that strains *D* and *E* had common origins (in a group of hibernating females). Unless otherwise shown the matings were all brother-sister matings.

DISCUSSION OF RESULTS

In general, these results show that selection from uninfected mothers tends to cause the percentage of individuals in the progeny which will become infected, to be reduced; and that selection from infected mothers rapidly increases the percentage of individuals susceptible to infection. Since males do not normally bite—and in my own experience do not become infected when fed artificially on infective blood—they remained an unknown element in the problem and hence prevented the ready determination of the recessive character and its subsequent isolation in a strain homozygous for it. Lines *A*, *B*, and *C* had their origins in the same lot of mosquitos

which were fed on an infected bird and then dissected. Line *A* was continued five generations, during which the inbreeding was all brother-sister, and the selection made from uninfected mothers. The percentage of infected individuals in any generation was never more than 13, and the average percentage of infected individuals was only 8.6. Line *B*, at this writing, has passed through ten generations. As can be seen from the tables and figures, this line was entirely negative to infection during the third, fourth and fifth generations. That these mosquitos—a total of 110—failed to become infected because of low infections in the birds is entirely disproved by the fact that mosquitos from stock lots fed on these same birds became *severely* infected. Plate VII shows an infection which was typical of the controls. Line *B* was closely inbred for the first four generations, but at this stage the numbers in each lot became so small that they had to be placed together in order to save the strain. This, however, was followed by the unfortunate result that in the sixth generation there was a reappearance of a fairly large percentage (24 per cent.) of infected individuals. Discontinuance of this mass selection and return to close inbreeding was followed by the result that the percentage of infected individuals decreased to 15 in the seventh, and to 3 in the eighth generation. The 20 per cent. in the ninth generation is based upon too small a number to have much significance. The average percentage for the whole ten generations was 7.4. A comparison of this figure with the percentage of individuals which became infected when a mass lot of this species was given an infective meal is interesting at this point. Out of 160 random feedings from controls, using females from larvae taken in the field, 45, or 28.1 per cent., became infected.

The results for lines *C*, *D*, and *E* which were selected from infected mothers were strikingly different. After the first generation of selection the lowest percentage of infected females in any generation was 21, and in one case the percentage went as high as 91. The average percentages of infected individuals were 50.0, 67.8, and 59.8, for strains *C*, *D*, and *E* respectively. These averages are strikingly higher than the percentage of infected individuals found in mass feeding, 28.1.

We find, then, that the number of individuals which become infected in a given generation is closely correlated with the pedigree of

that stock rather than upon differences in temperature or humidity conditions. It seems difficult to explain these results except by the assumption that hereditary characters determine whether or not a given individual mosquito will become infected when environmental conditions are favourable. (It is well known that low temperatures prevent the development of the parasites in susceptible mosquitos.)

A proof that there is operating within a species known to be susceptible to malaria an hereditary factor which determines the susceptibility or non-susceptibility of the individuals of that species will, of course, explain readily the phenomenon described previously (1927) as 'individual immunity.' On the other hand, this demonstration would seem to fit equally well in an explanation of the mechanism of natural immunity which involves the operation of an active resistance to infection on the part of the mosquito or in an explanation which shows that failure on the part of the mosquito host to become infected was due to a lack of some essential nutritive agent in its tissues. It is as easy to think of the one as being hereditary as it is of the other. In other words, a demonstration that natural immunity is hereditary in nature fits as well with the hypothesis that this immunity or non-susceptibility is active, as it does with that which assumes that it is passive or atreptic.

These results ought to warn us against assuming that because one insect has been infected by a particular parasite, all members of that species of insect are susceptible to infection. There are very likely many other cases in which only a small percentage of individuals of a species is susceptible to infection with a particular parasite and, under conditions favourable to selection, this percentage might fall to zero or rise to one hundred. We know of at least one other case in which this was true. While working upon the pébrine of silkworms, a protozoan disease caused by *Nosema bombycis*, Pasteur (1870), found that although the majority of the individuals succumbed to the infection, a few of them survived. From these individuals which survived he started resistant stocks—a procedure which was simpler than the one recorded in this paper because he was dealing with a disease of the larvae and was, therefore, able to get adults of both sexes which had resisted the infection.

If similar conditions obtain in human malaria, a possible explanation is offered of the innocuousness of a species in a malarious district

which is a dangerous carrier of malaria in another district. Such a species might be represented in the former district only by a strain composed of a low percentage of susceptible individuals; or it is conceivable that a race of non-susceptible mosquitos might arise in nature from a species known to contain susceptible individuals. The following examples are given as cases in which the existence of biological races differing perhaps only in their susceptibility to infection might be the explanation of the apparently innocuous character of these species.

Covell (1927) has collected the data recorded regarding different species of *Anopheles* and one of his paragraphs is here quoted in its entirety:—

‘One of the points brought out by the examination of the results recorded by various observers is that a species which has been proved to be an efficient natural carrier in one situation sometimes does not appear to play an important part in another. An example of this is afforded by *A. aconitus* in the Dutch East Indies. This species has been proved to be a good carrier of M.T. parasites experimentally, and has been found naturally infected both in Malaya and in Western Java. In the latter region a sporozoite rate of 7 per cent. was recorded by Winoto. Yet in another part of the Dutch East Indies, Swellengrebel and S. de Graaf dissected over 1,000 specimens with negative results, although in the same villages in which these were caught, not only *A. ludlowi* but also *A. sinensis* (*byrcanus*), *A. barbirostris* and *A. indefinitus* (*ragus*) were found to be naturally infected. The reason for this phenomenon has not been explained, and one can only surmise that in the latter region the conditions were in some way unfavourable for the development of the parasites in the former species.’

Another case differing principally because it rests upon epidemiological evidence but, nevertheless, being as difficult to explain, is given in the following personal communication by Dr. Paul F. Russell. He says:—

‘At the Norton hydro-electric scheme in Ceylon, in October, 1925, *Anopheles maculatus* mosquitoes were found breeding in abundance. Approximately a thousand coolies had been working there for about a year and were housed at the site. They had been recruited from various parts of Ceylon so that among them were many gametocyte carriers. Yet an examination of the hospital records showed that there had been no outbreak of malaria. In Malaya such a situation is not conceivable. Wherever numbers of coolies have been housed near *A. maculatus* breeding places there have always been sharp outbreaks of malaria, so that now no project is undertaken without preliminary and coincident *A. maculatus* control. For example, the new Singapore water supply project at Gunong Pulai, in Johore, is protected by extensive sub-soil draining, etc.’

In the light of what is reported here for bird malaria and *Culex pipiens*, it seems desirable to examine such cases in the future with this in mind.

On the other hand, it is no more unreasonable to assume that a species of mosquito now believed never to become infected with human malaria—any Culicine, for example—might possibly give rise to a strain which would be susceptible. If, in such a species, 'susceptibility' behaves as a recessive character, it might be expected to crop out only very rarely. We know that albino rats or crows occur very infrequently in nature, and that when they do appear they meet a life filled with vicissitudes which tend to exterminate them. However, if a Culicine mosquito appeared which was capable of becoming infected with human malaria, it is not likely that this character would in itself be inimical to the existence of the species, and hence it would be much more likely to remain in the hereditary constitution of the species than albinism would be to remain in the hereditary constitution of a rat or crow in nature. And, if such an individual happened to be one among the few which survived a winter or a drought in a particular region, it is certain that the number of susceptible individuals in the race which followed would be greatly increased. Following these facts and this line of reasoning, it ought not to be startling to us if we should find sometime a case in which human malaria is transmitted by Culicine mosquitos. Bruce Mayne (1928) has shown that an Anopheline mosquito may become infected with bird malaria. There is no evidence at present which would preclude or even render unlikely the possibility that if a sufficiently large number of Culicine mosquitos—perhaps hundreds of thousands—were fed upon human gametocytes, one of them would become infected. I do not wish to speculate upon the matter. However, it seems to me that we have enough evidence in hand to cause us to look upon the susceptibility and non-susceptibility of mosquitos to malarias with less faith in the immutability of their specificity.

SUMMARY

Selection in *Culex pipiens* in respect to its susceptibility or non-susceptibility to *Plasmodium cathemerium* (an avian parasite) has brought strong evidence in favour of the existence of 'susceptible' and 'non-susceptible' races in this species. Selection of progenies from infected mothers caused the number of infected individuals in a

particular line to increase rapidly in percentage. Selection from uninfected mothers caused a rapid decrease in the percentage of infected individuals in such a line.

It is believed that this proof weakens somewhat our conception that specificity is immutable. The proof that susceptibility and non-susceptibility behave as hereditary characters within a species, opens the question of whether a so-called susceptible species may not be capable of engendering a non-susceptible race, and conversely the question—even more important—of whether a so-called non-susceptible race may not be able to produce a susceptible race.

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EXPLANATION OF PLATE VI

Diagrammatic representation of the effects of selection upon susceptibility. The arrows indicate whether the selection was from infected or uninfected females. The number of arrows indicates the number of females which laid viable eggs. Designations of generations and lines same as Figs. 1 and 2.

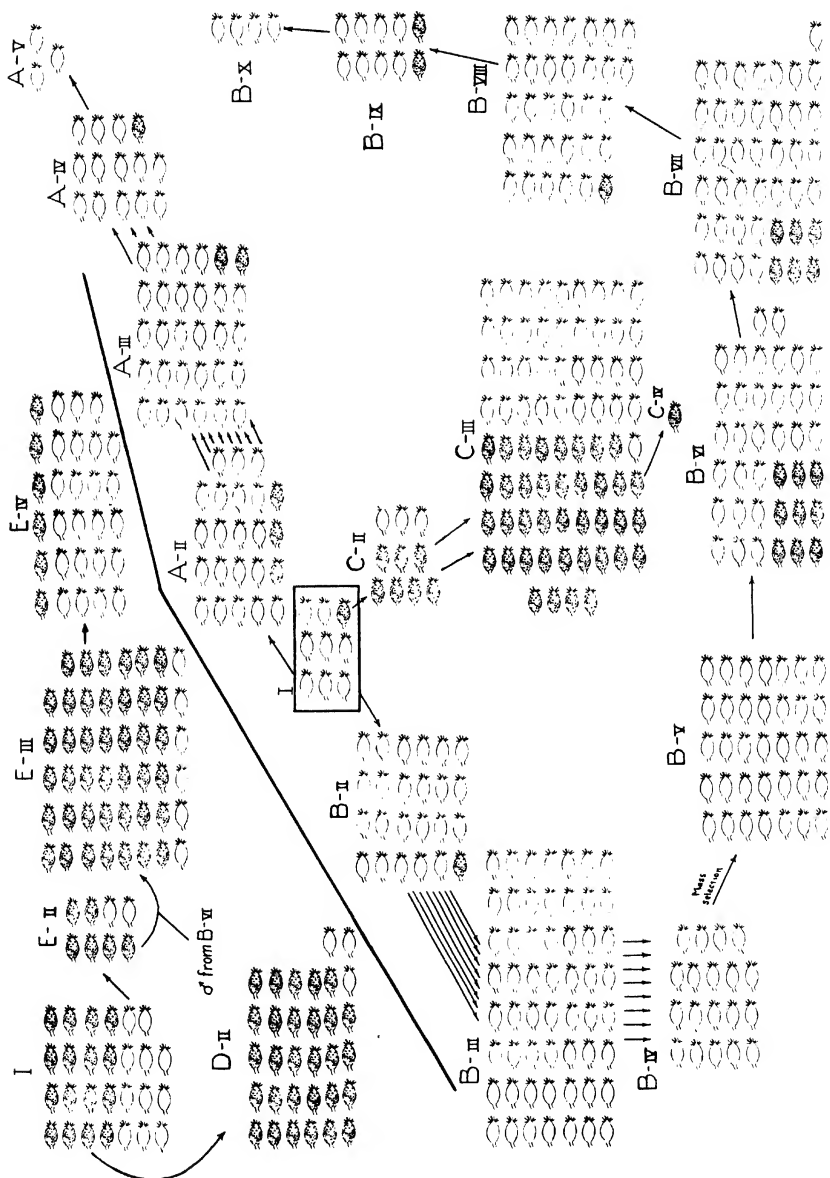
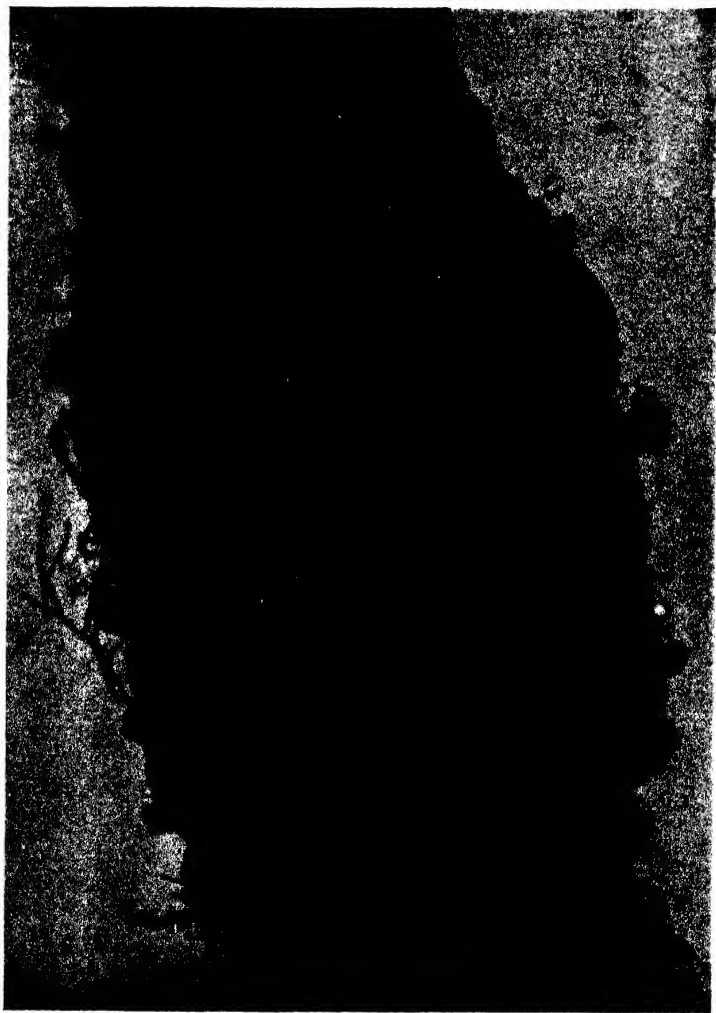


PLATE VII

EXPLANATION OF PLATE VII

Stomach of a mosquito from a control lot used to prove the infectiousness of the blood of the bird.



TUNNEL RAT-TRAP FOR STORES AND SHIPS

BY

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(Received for publication 18 June, 1929)

PLATES VIII AND IX.

A difficulty experienced in South Africa in dealing with forage and grain stores and similar buildings, is that as a result of persistent trapping, the rats sometimes become exceedingly wary and 'knowing,' so that the ordinary types of trap—gin, breakback and funnel cage—after a time prove ineffective. Another difficulty is that it is usually impossible to lay or set traps in a store filled completely or almost so with forage, grain or similar produce, unless open spaces and access passages have been left when the store was being filled. Even when such stores are constructed on thoroughly rat-proof lines, they will usually, where there is movement of produce into and out of the store, soon be found to harbour rats among the contents unless continuous preventive measures are taken—such as trapping or the keeping of cats.

The Union Health Department at first studied the possibility of providing a permanent 'home for rats' in such stores, with conveniently-placed openings in the floor or walls, giving access to pipes or channels leading to fixed and permanent rat-proof chambers conveniently placed either inside or outside the building, and provided with facilities for shutting off at intervals the ingress or egress channels, opening the chambers and removing the contained rodents. Experiments with 'homes for rats' constructed on these lines were carried out, but proved disappointing; the system, moreover, could only be installed in new or radically reconstructed buildings, and entailed considerable cost.

Attention was then directed to devising some form of continuous-acting trap which could be easily and cheaply installed in existing stores, and left there indefinitely or for lengthy periods, even when

the stores was filled with produce—the rats entering it being led to a cage trap placed so as to be convenient for inspection and removal at intervals.

This plan was made possible by the success of Mr. W. G. Powell, the Department's Chief Rodent Inspector, in devising a simple and effective 'valve' which, when placed in a tunnel or run-way used by rats, allows them to travel in one direction, but bars their return. This 'valve' consists of a stout tin or sheet-metal box, open at both ends, measuring 7 inches long, 4 inches high and $2\frac{1}{2}$ inches wide, the space between it and the sides and top of the tunnel being closed up with wire netting, so that rats traversing the tunnel must pass through the 'valve.' Inside the box is a little wire trap-door $7\frac{1}{2}$ inches long and 2 inches wide, hinged at its upper end into the metal box an inch from the top, and with its lower end resting lightly on the floor of the box and making with it an angle of about 25 degrees (see Plate VIII). A rat entering this box at its upper end finds that the little trap-door lifts lightly on its back, and passes through the opening thus made; the trap-door falls back into position immediately the animal has passed through, and the rat cannot return.

The remainder of the 'tunnel trap' is an adaptation of Powell's double-funnel wire-netting cage trap, a number of these traps being arranged in series so that each opens into a common tunnel or passage—the whole being enclosed in a narrow wooden box made in detachable lengths, each 7 feet long, 10 inches wide and $9\frac{1}{2}$ inches high—each length having a 'valve' placed near the lower end of the 'tunnel.'

Each of these lengths has six openings along one side, these being 3 inches in diameter and placed close to the floor of the box. The openings are opposite the open ends of funnels in the wire-netting cage enclosed in the box, the cage measuring $8\frac{1}{2}$ inches wide by 8 inches high. Between the cage trap compartments in the cage is a bait chamber which has a removable sheet-metal tray, and, at its further end, opens directly into the 'tunnel' of the cage. At each end of the cage the tunnel ends in a small compartment extending the whole width of the cage and communicating by a 3-inch opening with a similar compartment in the next length of cage. The ends of each length are fitted with catches, so that it may be securely

joined end to end (with the end openings opposite each other) with the next length, or with the terminal cage trap, or the end opening in one length may communicate with the similar opening in the next length by means of a 3-inch metal pipe—which may be from 2 or 3 feet up to 20 or 25 feet long.

At the end of the series of lengths so connected is a single-funnel cage trap enclosed in a detachable wooden box with hinged lid, 18 inches long and of the same cross-sectional dimensions as the sections of the trap, namely, 10 inches by $9\frac{1}{2}$ inches external. The dimensions and details of construction of the cage lengths and the terminal cage trap are shown in Plates VIII and IX. The whole apparatus may be made by a handyman at the rate of about one 7-foot length a day, at a cost of a few shillings for materials. Mr. Powell has made a half-scale model for demonstration purposes, the working of which may be interestingly and convincingly shown with the aid of a few mice. When a rat (or a mouse, in the half-scale model) enters one of the openings in the side of a length, it finds itself in the wide end of a funnel which leads it into a cage compartment, out of which it can escape through either of two funnels into a bait compartment which opens directly into the tunnel at the back of the box. Once in the tunnel it soon passes through the 'valve,' is unable to return, and passes on into the next length—and so on into the terminal cage trap.

In actual trapping work, the boxed-in lengths are laid along one, two or three sides of the store, in the angle between wall and floor, with the openings towards the interior of the building. Suitable bait is placed in each tray. In a forage or grain store, water and vegetables such as potatoes, cabbage or lettuce leaves, melon-peel, fruit, etc., will usually prove most attractive; in buildings where no grain is kept, wheat, barley or oats will be suitable. If water is not near and easily available, a little in a tray will prove very attractive. Bacon-rinds and fish also make excellent bait. The lengths should be securely fixed end to end, with catches or screw-nails and wire, the terminal cage trap being fixed at the lower end of the series, and so placed as to be convenient for inspection and removal at intervals. Care should be taken to ensure that the cage lengths are so placed that the tunnel valves all open in the direction of the terminal cage trap. If desired or convenient, any two cage

lengths, instead of being fixed directly end to end, may be joined by a length of 3-inch piping flanged at both ends so that it can be securely fixed by means of screw-nails over the end openings in the cage lengths. Experience shows that rats travel readily along such pipes. The pipes need not be horizontal, but may be sloped so that the rats travel either upwards or downwards; in this way cage lengths in a first or second floor may be 'drained' to a cage trap in the ground floor, or rats entering cage lengths in a cellar or basement may be led up to the ground floor.

Trap systems of this kind have been used in forage and grain stores for over a year past, by the Union Health Department, in conjunction with the Railways and Harbours Administration and some of the large Municipalities, and have proved very convenient and effective, especially where, as already explained, other forms of trap have come to be ineffective. Even where most of the cage lengths are covered with forage or grain, so that the bait trays cannot be replenished, it has been found that the system continues to function; rats enter to explore, or to find safe nesting places, and soon find their way to the terminal cage trap.

A trap system of this kind could easily be adapted and applied to ships. The wire-netting would have to be made of some rust-proof material, and the cage lengths, instead of being cased in wood, should be enclosed with substantial steel plates, the top plate being bolted on and easily removable so that the cage lengths can be taken out and inspected at intervals. With such a trap system I feel confident that the rat population of a vessel could be effectively controlled and kept down to a minimum, and those remaining might even be made use of as 'sentinels.' In 1925, in connexion with the question of periodical fumigation of ships for rat destruction purposes, I reported as follows to the Committee of the Office International d'Hygiène Publique—then engaged on drafting the new International Sanitary Convention:

'It is considered that in designing new ships attention should be paid to rendering them as rat-proof as possible, and facilities should be provided for rat destruction. The Convention should be framed so as to encourage the provision of such facilities. If ships were constructed on rat-proof lines and provided with special chambers or spaces, easily accessible to rats, and where they could find attractive cover and food, but which could be easily shut off when desired and the rats therein caught and examined, any rats finding their way on board could be utilised as "sentinels" or detectors of plague infection.'

The tunnel trap should greatly facilitate the carrying out of the foregoing proposals. Unfortunately, no opportunity of trying-out the system on ships in South African waters has so far occurred. The system should preferably be installed in the ship during construction.

I hope that the matter will be taken up and the possibilities of the system thoroughly investigated by the British Ministry of Health or some other National Health Authority, acting in consultation and co-operation with the Board of Trade and Shipping Companies.

POWELL'S TUNNEL TRAP.

MATERIALS AND DIRECTIONS FOR CONSTRUCTION.

FRAME for cage and trap : No. 8 gauge galvanised wire.

WIRE-NETTING : $\frac{3}{4}$ -inch (for rats) of No. 19 gauge galvanised wire.

PRONGS made of pieces of 14 gauge steel wire, sharpened at one end and made to project 1 inch beyond the ends of the wire-netting of which funnel is made.

BINDING WIRE : No. 16 or No. 18 gauge galvanised.

TRAYS : No. 8 sheet galvanised tin.

BOXES for cage lengths and cage trap : Pine boards, $\frac{1}{2}$ inch (or $\frac{3}{4}$ inch if desired ; dimensions given on plans are for $\frac{1}{2}$ inch boards) ; two hinges, hasp lock (for cage trap) ; Screws (for fixing top of cage lengths) ; Catches (for fixing cage lengths end to end).

CAGE LENGTHS : 7 feet long by 10 inches wide and $9\frac{1}{2}$ inches high (for $\frac{1}{2}$ inch boards). Each length has six entrance holes—each 3 inches diameter, and placed $\frac{1}{2}$ inch above level of floor of box.

FUNNELS :

In Cage Trap : Length, 12 inches ; Diameter of aperture at base, 8 inches ; Diameter at end of wire-netting (1 inch from end), 2 inches ; Diameter at end of prongs (which project 1 inch beyond wire-netting), $1\frac{1}{2}$ inches.

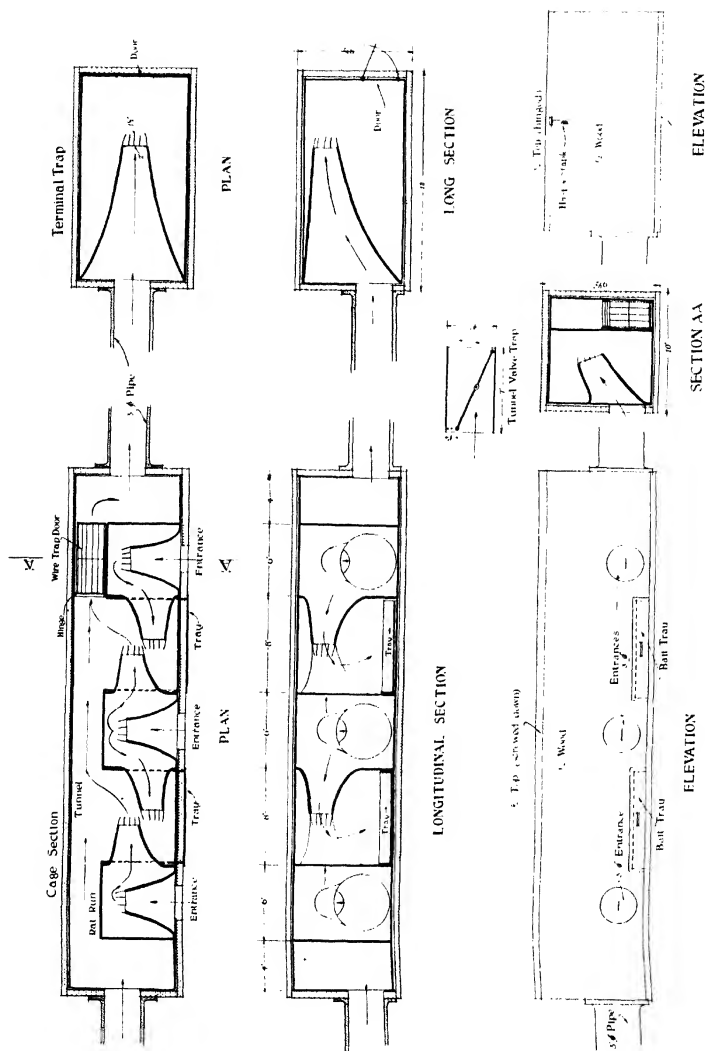
In Trap Compartments : Length, $\frac{1}{2}$ inches ; Diameter of aperture at base, 5 inches ; Diameter at end of wire-netting, $2\frac{1}{2}$ inches ; Diameter at end of prongs, 2 inches.

In Tray Compartments : Length, 5 inches ; Diameter of aperture at base, 5 inches ; Diameter at end of wire-netting, 2 inches ; Diameter at end of prongs, $1\frac{1}{2}$ inches.

In Tray Compartments and Terminal Cage Trap, end of funnel should be carried up *close to the roof of the compartment*.

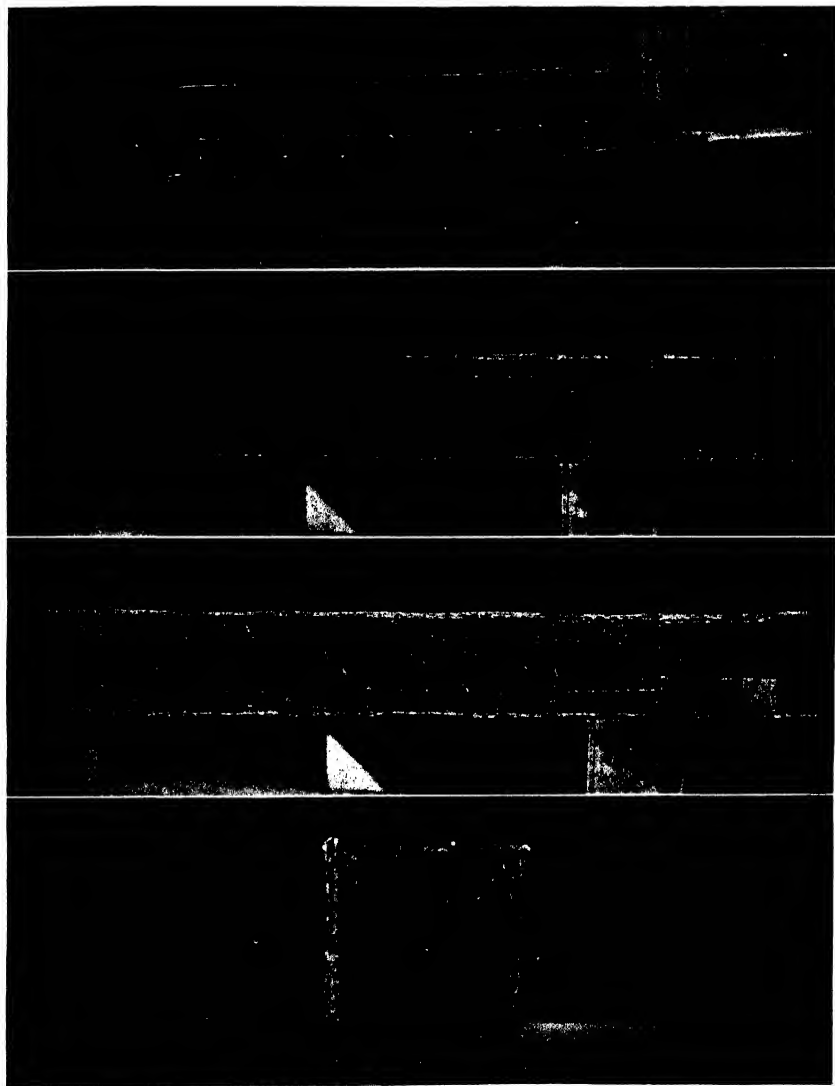
EXPLANATION OF PLATE VIII

Plan of Powell's Tunnel Trap. Scale: 1 inch to 3 feet (approximately).



EXPLANATION OF PLATE IX

Illustrating construction of Powell's Tunnel Trap.



THE DISTRIBUTION OF BLACKWATER FEVER IN NORTH AMERICA*

BY

J. W. W. STEPHENS

(Received for publication, 6 July, 1929)

TWO MAPS

UNITED STATES.

Locality and Date	Cases	Authority
<i>General</i>	'An article appears in the "Medical News and Hospital Gazette" (? 1832) of New Orleans, by an unknown author, in which he dates a case of haematuria as far back as 1820 and in his remarks on that and other cases, says: "Whether haematuria, more than epistaxis menorrhagia or any other of the bloody profluvia be the result of that bugaboo miasmata is questioned."'	Stamps (1886).
1820		
<i>Arkansas, Illinois, Indiana</i>	'... as early as 1837 and up to 1843, I encountered and treated many cases of this disease in the Wabash and White River bottoms in the States of Indiana and Illinois, and from 1843 to 1846 in the White River lowlands in the State of Arkansas, and that in each of these localities, where I practised at the periods named, I found this disease more or less prevalent every year.'	Day (1885).
1837		
1843		
1846		
<i>General</i>	'The writer confidently asserts that before the year 1850, the cases of haematuria, having the remotest connection with malarial disease, may be counted upon one's fingers; whereas since that date, it has appeared almost simultaneously from the Western boundaries of our country to the distant islands of Eastern Africa as a sporadic, endemic and epidemic disease.'	Manson (1886).
1850		
<i>Alabama</i>	'Dr. R. T. Michel, an able and eminent physician of Montgomery, Alabama, calls it a malignant malarial fever, gives an admirable and accurate description of the symptoms yet, strange to say, he dates the history of the disease from the year 1867.'	Smith (1900).
1867		

* STEPHENS, J. W. W. (1927). The distribution of blackwater fever in Europe. *Ann. Trop. Med. and Parasitol.*, **21**, 467.

— (1928). The distribution of blackwater fever in South-West Asia. *loc. cit.*, **22**, 53.

— (1928). The distribution of blackwater fever in India. *loc. cit.*, **22**, 170.

— (1928). The distribution of blackwater fever in Burma and the Far East. *loc. cit.*, **22**, 179.

— (1929). The distribution of blackwater fever in Africa. *loc. cit.*, **23**, 67.

UNITED STATES—*continued.*

Locality and Date	Cases	Authority
Louisiana	'In 1886, Dr. R. H. Day read a paper before the State Medical Society, at Baton Rouge, Louisiana. . . . The literature of haemorrhagic malarial fever is very meagre and dates back only a few years in the past.'	
Louisiana 1843	'The disease was observed by a number of physicians many years before the close of our civil war. Among others, I may mention Dr. C. Glidden Young, of Louisiana, 1843.	
Alabama 1845	Dr. T. H. Anderson, of Mobile, Alabama, was acquainted with the disease prior to 1850. Dr. A. G. Mabry. . . . said in 1870. . . . More than twenty-five years ago I treated in the vicinity of Selma, Alabama, cases of intermittent fever presenting in a marked degree all the symptoms characteristic of these cases at the present day.'	
Alabama, Arkansas, Mississippi, North Carolina, Texas, 1866	'In Texas it showed itself first in 1866 (Ghent, Tate, Starley, Hewson, Johnson (II), Heard), and about the same time on the coast and in the central swamps of Mississippi as far up as Natchez (Sharpe, in the Transactions of the Mississippi State Med. Soc. for 1874), in Arkansas (Duval, in the Transactions of the Arkansas State Med. Soc. for 1871) and in Alabama, where it is very prevalent to a very considerable extent according to accounts by Kinnard, Scholl, Osborn, Michel, Riggs, Hendrick, Weatherley, Anderson, Mabry and Webb; and it has lately been reported to occur also in North Carolina, by Raleigh and Greene.'	Hirsch (1883).
New Jersey, North Carolina, Pennsylvania	'The disease,* being comparatively rare in this latitude (Pennsylvania), is sometimes overlooked on this account. 'Of the seven cases which I have noted during fifteen years, five originated in Pennsylvania, one in New Jersey, and one in North Carolina.'	Tyson (1883)a.
Alabama	'Two degrees of the disease are met with, a milder form in which other symptoms as well as the haematuria are less pronounced and of which instances occur in the Middle States, as well as the South and West of this country. Of this kind seem to be the cases studied by Harley and other English physicians. In addition to this, there is a second more malignant form attended by great prostration, vomiting and yellowness of the skin, along with copious discharges of bloody urine. Instances of the latter are numerous in the Southern States of this	

* i.e., the milder form, possibly cases of paroxysmal haemoglobinuria.

UNITED STATES—continued.

Locality and Date	Cases	Authority
<i>Alabama—continued</i> 1868	country. . . . My attention was first called to it in September, 1868, when I received specimens of urine and the history of some cases from Dr. R. D. Webb, of Livingston, Ala. . . .	
<i>General</i> , 1869	'Haemorrhagic malarial fever. . . . Syn. <i>Haemorrhagic Malarial Fever</i> [Michel]. <i>Black Jaundice</i> [Chent]. <i>Cachemia</i> [Osborn]. <i>Cachemia Haemorrhagica</i> [Owens]. <i>Icteroide Pernicious Fever</i> [McDaniel]. <i>Malignant Congestive Fever</i> [Osborn]. <i>Purpuraemia</i> [Riggs.] <i>Yellow Remittent</i> [Sholl]. <i>Yellow Disease</i> . <i>Cane-brake Yellow Fever</i> . <i>New Disease</i> .'	Boston (1869).
<i>Alabama, Georgia, Mississippi, Texas</i>	Hare (1892) sent out questions to physicians residing in the areas marked in the census as having a death-rate from malaria of seventy per thousand or over (presumably those in the margin as no returns from any other areas are recorded). Fifty-four of the one hundred and fifty-five (physicians) see it frequently. The list of remedies for malarial haematuria used by the one hundred and seven physicians who sent replies to questions sent out to physicians residing in the areas marked in the census as having a death-rate from malaria of seventy per thousand or over.	Hare (1892). Hare and Krusen (1895).
<i>Pacific Coast (Malaria)</i> ...	Only the milder forms of malarial fever prevail on the Pacific Coast (Washington, Oregon, California). (Cp. under heading California.)	Perry (1898).
<i>Alabama, Arkansas, Florida, Louisiana, Mississippi</i>	Haemoglobinuria from malarial disease is frequently observed in Alabama, Mississippi, Louisiana, Arkansas and, to a less extent, in Florida.	Weber (1901).
<i>Alabama, Arkansas, Florida, Georgia, Mississippi, N. Carolina, S. Carolina, Tennessee, Texas, Virginia</i>	In North America, haemoglobinuric fever is found in the Southern States, especially parts of Texas, Arkansas, Mississippi, Tennessee, Alabama, Georgia, Florida, N. Carolina, S. Carolina, Virginia.	Deaderick and Thompson (1916).
<i>General (Malaria)</i> ...	Trask states 'that at one time malaria was endemic over a much greater area of the United States than it is to-day, and in many sections where it is still endemic its prevalence has greatly diminished. Fifty years ago the disease prevailed farther north than it does now. The endemic area extended to the great lakes and into Canada. Ague was in this section the most common of ailments and quinine the most universal of household remedies. The early literature indicates that the disease was formerly more	Trask (1916).

UNITED STATES—*continued.*

Locality and Date	Cases	Authority
<i>General (Malaria)—contd.</i>	<p>or less prevalent also in Iowa, Minnesota, the Dakotas, Utah, Colorado, Montana and Wyoming.</p> <p>'It would be of interest to explain satisfactorily why it has all but disappeared from Wisconsin and Michigan, two States at one time badly infected, and still persists in certain sections of New England.'</p> <p>'At the present time, there are three principal well-recognised endemic areas, one large area and two smaller ones. The large endemic area covers the whole South-Eastern portion of the United States—the territory extending from the Gulf of Mexico to a line north of the Ohio River, and from the Atlantic seaboard to and into the Eastern part of Kansas, Oklahoma and Texas. Of the two smaller endemic areas, one includes a section of the Northern part of New Jersey, South-Eastern New York, Connecticut, Rhode Island and part of the State of Massachusetts. The third recognised endemic area is in California, and includes the Sacramento and San Joaquin valleys.'</p> <p>'Fort Washington, Maryland, had for several years up to 1913 the highest malaria sickness rate of any (army) post in the United States. In 1914 the highest rate (73 per 1,000 mean strength) was at Washington Barracks, in the district of Columbia. The second highest was at Fort Myer, Virginia, just outside of Washington. . . . and the third highest at Leavenworth, Kansas.'</p>	
<i>General</i>	<p>'We know now that it is endemic. . . . in . . . certain of our Southern States. . . .'</p> <p>'It would seem reasonable to consider black-water fever in the United States as secondary to the malignant tertian infections brought in by the slaves.'</p>	Stitt (1928).

ALABAMA.

Locality and Date	Cases	Authority
<i>Alabama</i>	<p>'Two degrees of the disease are met with—a milder form in which other symptoms as well as the hæmaturia are less pronounced, and of which instances occur in the Middle States, as well as the South and West of this country; and second, a more malignant form attended by great prostration, vomiting, etc., yellowness of the skin, along with copious discharges of bloody urine.'</p>	Tyson (1883)6.

Locality and Date	Cases	Authority
<i>Alabama—continued</i>		
1868	'While a majority of cases of malarial haematuria are intermittent many are continuous, and of my seven cases, only two were distinctly intermittent. One of these cases I published in a clinical lecture in the <i>Philadelphia Medical Times</i> , as far back as September 1, 1871.'	
1863	'My attention was first called to it in September, 1868, when I received specimens of urine and the history of some cases from Dr. R. D. Webb, of Livingston, Alabama, who wrote also that it was not known in that part of his State, at least prior to 1863 or 1864.'	
<i>Montgomery</i> 1869	Deaderick and Thompson (1916) state that in March, 1869, Dr. R. F. Michel, of Montgomery, Ala., read a paper before the Medical Association of the State of Alabama, in which he spoke of the disease 'as a malignant, malarial fever following repeated attacks of intermittent, characterised by intense nausea and vomiting, very rapid and complete jaundiced condition of the surface as well as most of the internal organs of the body, an impacted gall bladder, and hemorrhages from the kidneys. These phenomena presented themselves in an almost uninterrupted link, attended by remissions and exacerbations. It is a fever peculiar to the United States.'	Deaderick and Thompson (1916). Michel (1869).
	A malignant malarial fever following repeated attacks of intermittent, characterised by intense nausea and vomiting, very rapid and complete jaundiced condition of the surface as well as most of the internal organs of the body, an impacted gall bladder, and hemorrhages from the kidneys.	American (1870). Michel (1869).
	These phenomena present themselves in an almost uninterrupted connection, attended by remissions and exacerbations: the disease is one peculiar to the Southern States. It has received various synonyms as Black Jaundice; Cachemia Haemorrhagica; Malignant Congestive Fever; Icteroide Pernicious Fever; Purpuraemia; Yellow Remittent; Yellow Disease; Canebrake yellow Fever, etc.	
<i>Greensboro</i> 1867	Deaderick and Thompson (1916) state that 'in 1867, Dr. T. C. Osborn (1868, 1870), of Greensboro, Ala., observed ten cases, five of which ended fatally, some with anuria and uremia. All the patients had been repeatedly attacked with malaria. A few months later his son, Dr. J. D. Osborn (1869) read a paper before the Greensboro Medical	Deaderick and Thompson (1916). Osborn (1868). Osborn (1869). Osborn (1870).
1869		

ALABAMA—continued.

Locality and Date	Cases	Authority
<i>Greensboro—continued</i>	Society, from which it is evident that the disease was becoming more prevalent and that the country people were regarding it as yellow fever.'	
<i>Selma</i> 1870 1845	Deaderick and Thompson (1916) state that Dr. A. G. Mabry, in a report of a case of intermitting icterode haematuric fever . . . in 1870, says, 'It is a mistake to suppose that this is a new form of disease. More than twenty-five years ago I treated in the vicinity of Selma, cases of intermitting fever presenting in a marked degree all the symptoms characteristic of these cases at the present day.'	Deaderick and Thompson (1916). Mabry (1872). Mabry (1870).
<i>Camden</i> 1874	Deaderick and Thompson (1916) state that 'Dr. McDaniel, of Camden, Alabama, described hemoglobinuric fever in 1874, and says, "In calling up my own reminiscences, I am sure that I have occasionally, ever since my boyhood, seen isolated cases of what was considered intense bilious fever, with the surfaces and under tissues stained deeply yellow and with the urine deep red. They were nearly all fatal and were called in older phrase, " "bilious congestive," " and in more recent " "pernicious bilious." " I have also but more rarely known groups of similar cases associated, say three or four cases occurring on the same premises or in the same family, about the same time. All such cases in addition to the deep so-called bilious colour and the red urine, had jactitation, suspirous breathing, inordinate thirst and vomiting of various shaded and tinted so-called bilious matters. By diligently enquiring I have ascertained that very many old physicians, some of whom have now retired from practice, are satisfied that they have observed similar cases, sometimes singly and sometimes in groups." '	Deaderick and Thompson (1916). McDaniel (1874).
<i>Camden</i> 1867-1882	'All the eighteen cases . . . were of the type referred to in Dr. James Tyson's paper as <i>malignant haematuria</i> , called in Alabama, hemorrhagic malarial fever, and occurred in the writer's practice from 1867-1882 inclusive.' I now present a table of 178 cases of hemorrhagic malarial fever made up of : 25 cases seen by myself 33 reported by Dr. Webb, of Livingston, Ala. 22 reported by Dr. Saml. Perry, of Marion, Ala. 46 reported by Dr. Jackson, of Greene Co., Ala.	McDaniel (1883).

ALABAMA—continued.

Locality and Date	Cases	Authority
<i>Camden—continued</i>	<p>11 reported by Dr. Webb, of Greene Co., Ala.</p> <p>41 reported by Dr. Minor, of Greene Co., Ala.</p> <p>Of these 178 cases :</p> <p>85 took quinine (as part of the treatment).</p> <p>93 did not take quinine (as part of the treatment).</p> <p>Of the 85 cases with quinine :</p> <p>35 or 41 per cent. died.</p> <p>Of the 93 cases without quinine :</p> <p>16 or 18 per cent. died.</p>	
<i>Falkland</i>	A statement of 41 cases (1865-1883)	Minor (1883).
<i>Livingston</i>	Analysis of 33 cases.	Webb (1883).
	<p>* In my account of the epidemic fever which prevailed in 1883, in Brewton, a small town in Escambia County, Alabama, I have stated in some detail the problem of differential diagnosis which had to be solved there. I had for many years been familiar with yellow fever and to me it seemed perfectly clear that the fever at Brewton was yellow fever. The physicians at Brewton had been for many years familiar with haemorrhagic malarial fever and they asserted with persistent emphasis that the Brewton fever was haemorrhagic malarial fever. . . .'</p> <p>* In the fall of 1884 it came to my knowledge that cases of haemorrhagic malarial fever were recurring in several counties of Alabama. . . .'</p>	<p>Cochrane (1884).</p> <p>Cochrane (1885)a.</p>
<i>Lowndes County</i>	<p>* As the result of this effort I obtained : (1) Clinical reports of three cases, two from Dr. S. Hopping, of Letohatchie, in Lowndes County, Alabama, and one from Dr. R. D. Webb, of Livingston, in Sumter County, Alabama, which I gladly include in my paper ; (2) Several specimens of the characteristic red urine. . . .'</p>	
<i>Sumter County</i>	<p>* My collection of cases numbers six hundred and forty-two (642)—four hundred and eighty-four recoveries (484) and one hundred and fifty-eight (158) deaths.' (Forty-four replies were received in response to one thousand letters issued among the physicians of Alabama).</p> <p>* The percentage of deaths to cases, therefore, is 24.66 ; or, in round numbers, one-fourth of the cases died, and three-fourths of them recovered.'</p> <p>* <i>Black Vomit</i>.—I took a great deal of trouble to secure full accounts of black-vomit cases ;</p>	

ALABAMA—continued.

Locality and Date	Cases	Authority
<i>Sumter County—continued</i>	<p>but out of the six hundred and forty-two cases reported, only fourteen presented this symptom and in only three cases is the black vomit reported to have resembled coffee grounds. It is a curious fact, too, that those of my correspondents who have practised in the most intensely malarial sections of the State, and who have seen the largest number of cases of the most malignant types, have not seen black-vomit cases at all; and some of them distinctly take the position that the so-called black vomit of haemorrhagic malarial fever is always altered bile and not like the black vomit of yellow fever, altered blood.'</p> <p>'<i>The Red Urine.</i>— . . . I consider that Dr. Sternberg's researches have definitely settled this question . . . —the fact, namely, that the red or dark discoloration of the urine is due to the presence, not of blood, but of the blood pigments and especially haemoglobin—is also fully established for the haemorrhagic malarial fever of Alabama.'</p>	
<i>Selma</i>	<p>'About 1.30 o'clock on the morning of Dec. 15, 1885 This young man had many attacks of this dangerous malady since childhood, this being the third attack since August last, 1885. He was one of those in whom quinine always caused "hemorrhage from the kidneys" (?). Therefore I had never been able to use the bark derivatives. . . . His was thoroughly a case of malarial cachexy: he had a spleen as large as a peck measure, hard, sore and extending down to the crest of the left ilium and to the right of navel.'</p> <p>'I have seen four cases where no quinine was taken prior to the hemorrhage. . . . Ten successive cases without a death.'</p>	<p>Riggs (1886).</p> <p>Du Bose (1899).</p>
<i>General</i>	<p>Hare (1892) records that 'In six hundred and forty-two cases collected by Jerome Cochrane, health officer of Alabama, from different practitioners, there were one hundred and fifty-eight deaths, the death-rate being about twenty-five per cent. . . .'</p> <p>'Dr. T. W. Ayres, of Jacksonville, Ala., calls my attention to the fact that the Report of the State Board of Health of Alabama, shows that in 1887, 1888, and 1889, there were thirty-nine deaths from malarial haematuria, of which twenty-five were males and fourteen females. And he also records that, of one hundred and eight cases, seventy-three were males and thirty-five females.'</p>	<p>Hare (1892).</p>

ALABAMA—continued.

Locality and Date	Cases	Authority
<i>Union Springs</i>	'Twenty-five years ago the so-called hemorrhagic fever or malarial hematurlia was one of the most frequent and fearful diseases in this county, but since then it has been gradually disappearing until it is one of the rarest diseases. So far as I can learn, there have been only two or three cases in the county within the past eight years. I have had the good fortune to have seen only four cases, all of which occurred nine years ago—during my first year of practice.'	Harris (1904).
1867	'Haemorrhagic malarial fever as a distinct disease was unknown to the physicians of Alabama prior to 1867. True enough, there were, scattered here and there, a few cases at a much earlier date, but it was regarded by the physicians of that day as a malignant form of malarial fever. It did not attract much attention until the year 1867, or perhaps a little earlier, when it prevailed to an alarming extent along the water courses of the Southern States. Almost every community within the area was afflicted with the terrible scourge.'	Brockway (1912).
<i>Jackson</i>	7 (1920-4)	Barber (1926).
<i>Montgomery</i>	2 (1923-4)	
	'In 1922 I was in Southern Alabama . . . and we heard of no cases of blackwater.'	

ARKANSAS.

Locality and Date	Cases	Authority
<i>Fort Smith</i>	Deaderick and Thompson (1916) state that: 'In Arkansas, hemoglobinuric fever was first recorded by Dr. E. R. Du Val, of Fort Smith, in a paper read before the State Society, in 1871. He believed the case he recorded to be the first to occur in the State.'	Deaderick and Thompson (1916). Du Val (1871).
1871		
<i>Monroe County</i>	Deaderick and Thompson (1916) state that, 'In 1880, Dr. G. B. Malone, in Monroe County, Arkansas, reported 155 cases met in his practice.'	Deaderick and Thompson (1916). Malone (1880) and (1881).
<i>South-West Arkansas</i>	'In 1873, I commenced the practice in South-West Arkansas, a very sickly, malarious country, and so as chills were common, I found that this disease was only the result of aggravated chills or protracted cases of malarial poison. The doctors had never cured a single case. . . . Well, I cured that year eight out of nine on this same experimental treatment.'	Weathers (1886).
1873		

ARKANSAS—*continued.*

Locality and Date	Cases	Authority
Wallaceburg	2 cases (brother and sister) (1885)	Stamps (1886).
Batesville	'Haemorrhagic malarial fever. It is always considered dangerous. I have never seen a case of it in the infant, and very seldom do we see a case in our part of the State, either in the adults or the children, however, occasionally in both.'	Lawrence (1887).
Wallaceburg	'The majority of the cases that prove fatal occur in rural districts, where autopsies are looked upon as unpardonable intrusions upon the dead.'	Stamps (1888).
General	'A resident of Arkansas for the past five years, the latter two having been spent on the banks of the Red River.'	Woldert (1895).
Camden	'The mortality from this disease is not so heavy as it was twenty years ago, and at that time it was treated almost exclusively by massive doses of quinine.'	Meek (1897).
Snyder	'The disease is quite common in the swamp countries of this State, where the pernicious forms of malaria are most prevalent.' 11 cases, 1 death (Feb., 1898-March, 1899)	Roop (1899).
Deckerville	'Out of 16 cases seen by me, 13 were males and 3 females, the oldest being 38 and the youngest 5 years of age. Dr. McElroy, of Stoval, Miss., shows a record of 40 cases, of these, there were 30 males and 10 females. . . . Of my 16 cases, 3 were very light mulattoes; of Dr. McElroy's 40 cases, 13 were negroes, 2 of which, he writes to me, were full-blooded negroes.'	Burns (1900).
Marianna	4 (1907)	Deaderick (1908).
Hot Springs	'Twenty-seven cases were seen in rural residents, and seven in townspeople. . . . All, without exception, had suffered previously with malaria, most of them repeatedly. . . . In thirty cases, quinine was being taken for malaria when the outbreak occurred. In four cases quinine could be absolutely excluded.'	Deaderick (1914).
Eastern Arkansas	Deaderick, in Deaderick and Thompson (1916) states that, 'It is probably becoming less frequent in some of the Southern States, judging from my experience in Eastern Arkansas.'	Deaderick and Thompson (1916).

ARKANSAS—*continued.*

Locality and Date	Cases	Authority
<i>St. Francis River Bottoms</i> ...	' Dr. E. G. Fulton, Iberia : " In the summer of 1906, as an under-graduate, I went over into the St. Francis River Bottoms in Ark., got as far from the rail-road as possible, and started in to practice medicine. I had only been practising about two weeks and thought I was getting along fine, when I was called to see a case of black-water fever.'	Wright (1917).

CALIFORNIA.

Locality and Date	Cases	Authority
<i>Sacramento</i>	' We very rarely have cases of malarial haematuria here.'	Briggs (1900).

COLORADO.

Locality and Date	Cases	Authority
<i>La Grange</i> 1868-9	' In our own country, malarial fevers, mild and severe, have prevailed since the first settlement and quinine has been extensively used in the treatment. But malarial haematuria is a comparatively recent disease. It was not known, or but rarely met with, until since the close of the Civil War. I saw the disease soon after its first appearance in the settlements along the Colorado river, near La Grange, in the years of 1868-9. . . '	McLaughlin (1904).
<i>Denver</i>	2 cases.	Brasher (1899).

FLORIDA.

Locality and Date	Cases	Authority
<i>General</i>	' From a rather extended experience with the disease in question, in the State of Florida, I am prepared to say that <i>all cases are amenable to treatment by quinine, if such treatment be entered upon within thirty-six hours of the onset. . . .</i> It occurs only in those persons who have been for a long time subjected to slow malarial infection without the proper administration of quinine . . . some superficial observers have named the disease "highland yellow fever." The distressing symptoms are haematuria, intense nausea and black vomit, extreme thirst, frequently repeated chills of a congestive character, and, at times, sinking turns amounting almost to	Stubbert (1886).

FLORIDA—continued.

Locality and Date	Cases	Authority
<i>General—continued</i>	syncope. . . . Dr. Maxwell has met with equally good results and in a large number of cases. . . . The position taken by some, that "pure blood" is passed from the kidneys, seems to me untenable. . . . Is it not more reasonable to suppose that the evacuations are urine coloured by the colouring matter of the red corpuscles. . . . ?	
<i>General</i> 1853	'G. Troup Maxwell, of Ocala, Florida, writes me, in 1883, that he first observed cases in Florida thirty years ago, and published an article on the disease in 1860.' [The possibility of confusion with paroxysmal haemoglobinuria in early cases and others should be borne in mind.]	Tyson (1886). Maxwell (1860).
<i>Leesburgh</i>	'This disease is getting to be a very common occurrence in Florida, Georgia, and the Mississippi bottoms. . . . Hemorrhagic fever attacks those who have previously had some form of malarial fever. . . .' Records 5 cases.	Bush (1896).
<i>Grenville</i>	'In a number of cases that I have seen I have been firmly convinced that the agent which wrought such terrific destruction with the blood elements was not the parasite itself, but some toxin, the existence of which depended either on the assistance or presence of the <i>Plasmodium</i> .'	Greene (1905).
<i>General</i>	'Finds that nephritis is present in all cases and influences the treatment.'	Ross (1916).

GEORGIA.

Locality and Date	Cases	Authority
<i>Dalton</i>	<i>Malarial haematuria.</i> —A unique case of this disease is recorded by Chas. P. Gordon, M.D., Dalton, Ga., in the July number of the <i>Atlanta Medical and Surgical Journal</i> 'As there are in congestive fever the cerebral, thoracic and abdominal varieties, he would add another, <i>the renal variety</i> , in which the congestive tendency is to the kidneys, with all the pernicious results following malarial haematuria.' (p. 418).	Gordon (1872).

GEORGIA—continued.

Locality and Date	Cases	Authority
<i>Americus</i>	Haemorrhagic malarial fever.—At a recent meeting of the Atlanta Academy of Medicine, Dr. Cooper, of Americus, Georgia (<i>Atlanta Med. and Surg. Journ.</i>), called attention to the value of large doses of the tincture of chloride of iron in the treatment of this variety of fever. Quinine seemed to be of little use in this disease. (p. 187).	Cooper (1872) ?.
<i>Albany</i>	'Hemorrhagic malarial fever.— . . . He also remarks as a peculiarity of the disease, that its ravages have been almost exclusively confined to what is regarded as the healthiest portion of the country—the pine woods.' (p. 396).	Cromwell (1872).
<i>Americus</i>	Deaderick and Thompson (1916) state that, 'the affection was first reported in Georgia, by Dr. W. A. Greene, of Americus, in 1872.'	Deaderick and Thompson (1916). Greene (1872).
<i>Macon</i>	'Concludes . . . that this disease is purely malarial.' (p. 140).	McHutton (1884 and 1885-86).
<i>Albany</i>	'My attention was first called to this disease in the summer of 1884, by having a specimen of urine referred to me for analysis. . . . I received many specimens (more from Dr. Hillsman, of Albany, Georgia, than from anyone else. . . .')	McHutton (1891).
<i>General</i>	'W. Shropshire reports two cases. . . . In one instance, quinine in moderate doses had been used in a severe case of malaria, and blood began to be passed in the urine. . . . Another similar case is offered to show that during the administration of moderate doses of quinine, hemoglobinuria may develop.'	Shropshire (1901).
<i>Colquitt County</i>	'A county health officer of an adjoining county, Colquitt, told me that in 1920 about 25 cases of blackwater had been reported in his county.'	Barber (1926).
<i>Mitchell County</i>	'My own observation is rather meager: in 1921 I knew of one case in Mitchell County.'	
<i>Leesburg</i>	'One of the local physicians in Leesburg, Georgia, claims to have treated blackwater fever, with very good results, by the administration of antistreptococcic serum.'	Boyd (1926).
<i>Lee County</i>	'In Lee County, Georgia, the former headquarters of this station (station for field studies in malaria) we were located in an area in which the endemicity was normally not high, but which is subject to severe visitations of epidemic malaria, at which time cases of blackwater fever were not uncommon.'	Boyd (1926).

ILLINOIS.

Locality and Date	Cases	Authority
<i>Elgin</i>	'W. M., male, age 25, recently from a malarial region of Kentucky and for years a victim of that disease. . . . Dr. Bell was called and found the patient in a delirious state, temperature 104°. . . . The Doctor prescribed quinine in large doses frequently, sponge baths and opium as a sedative. I was called a few hours later and found that the patient had passed a very large quantity of sanguineous urine. . . . he became very much jaundiced, the entire body being of an intensely lemon yellow colour.'	Gahagan (1895).
<i>Wetaug</i>	'Malarial hematuria.—Dr. L. M. Winsted, of Wetaug, read this paper. . . . The author narrated ten typical cases of the malignant type of the disease.'	Proceedings (1902).

KENTUCKY.

Locality and Date	Cases	Authority
<i>Jefferson County</i>	'In a practice above the average country practice around Lacona, Jefferson County, Ky., mostly in the first bottom of the Ohio River, extending through all the years, from 1847 to and through 1875, I have met with nine cases of malarial hematuria: had one death. . . . I met with no case unless one-half to three-fourths of the population were the subjects of malarial diseases, nor did ever more than one case in a single year occur with me, until the one that has passed, when I met with four.'	Foss (1876).
<i>Louisville</i>	Askenstedt, of Louisville, contributes a paper (presumably in reference to blackwater in Kentucky).	Askenstedt (1912).

LOUISIANA.

Locality and Date	Cases	Authority
<i>New Orleans</i> 1859	Deaderick and Thompson (1916) state that, 'Faget treated the disease as early as 1859, and states that the cases with hematuria and hematemesis had frequently been seen in New Orleans and been mistaken for yellow fever. Inasmuch as Faget considered hematemesis a common symptom of hemoglobinuric fever, it is possible that he himself confounded the two diseases in some instances.'	Deaderick and Thompson (1916), p. 220. Faget (1869). Faget (1870).

LOUISIANA—continued.

Locality and Date	Cases	Authority
<p><i>Monroe</i></p> <p>1859</p>	<p>Deaderick and Thompson (1916) state that, 'In the United States hemoglobinuric fever was first described by Dr. J. C. Cummings, of Monroe, Louisiana, in 1859. He reported six cases and refers to numerous cases during the previous season.'</p>	<p>Deaderick and Thompson (1916), p. 220. Cummings (1859-60).</p>
<p><i>Opelousas</i></p> <p>1867</p>	<p>'Billy Fox, aged thirteen years, had several paroxysms of chills and fevers. Quinine was prescribed by the parents on two occasions, which was followed immediately (both times) by haemorrhage from the mucous membrane of the urinary organs. I was called (August, 1867) to see the boy and . . . I prescribed ten grains of quinine to be divided in three doses, and to be given next day. . . . An hour after the third dose had been administered, the patient had a profuse haemorrhage from the urinary passages. My little patient was removed to Opelousas. . . . An eminent physician of that town was called in . . . and again quinine was prescribed and administered, and followed by the same kind of haemorrhage. . . . The boy is again under my treatment, suffering with chills and fever (tertian). Two weeks ago, his father prescribed and administered three or four doses of an infusion of <i>cinchona</i> and Virginia snakeroot, which was followed by haemorrhage of same organs. It has been my misfortune to have had another similar case last fall. To a little girl, seven years old, quinine was administered in different ways, but was invariably followed by haemorrhage of the urinary passages.'</p>	<p>Cacheré (1869).</p>
<p><i>New Orleans</i></p>	<p>'Two cases of what he calls "malarial haemorrhagic fever" in one of which he says, "the urine was loaded with albumen and showed, under the microscope, abundant blood corpuscles" and in the other, that the urine was "loaded with blood."'</p>	<p>Bemiss (? prior to 1885). Journal (1885).</p>
<p><i>Iberville Parish</i></p>	<p>'One of the complications which is beginning to be quite common in this section is haematuria.'</p>	<p>Owen (1885).</p>
<p><i>Baton Rouge</i></p>	<p>'It was my fortune, early in my medical career, to become familiar with this most terrible disease, both in its mild and most virulent forms.'</p> <p>'It is emphatically an hepatic and blood disease of malarial origin.'</p>	<p>Day (1886).</p>

LOUISIANA—continued.

Locality and Date	Cases	Authority
<i>General</i>	'From her swamps and bayous we draw our greater share of cases of typical malaria and all cases of haematuria.'	Ballard (1899).
<i>Lake Charles</i>	'My experience with malarial hematuria in private practice has been limited to nine cases, three of which died, six recovering entirely.'	Watkins (1901).
<i>Ruddock</i>	'Out of thirty cases treated by me, twenty were white (seventeen men and three women) and ten were negroes (eight men, one boy, one woman).'	M'Kay (1902).
<i>Raccourci</i>	'Of all the diseases with which the practitioner in the Mississippi Delta has to contend, for fatality none is equal to malarial haematuria. With a mortality of fully fifty per cent., it is not a matter of surprise that it is held in dread fear by the inhabitants of the region in which it prevails. . . .' 'Histories of my first four cases. . . .'	Rigney (1895).
<i>General</i>	'Quinine as a specific in malarial haematuria.—Dr. L. G. Le Beuf read a paper at the last meeting of the Louisiana State Medical Society, on the subject of quinine in haematuria, and reported four cases of the worst type of haematuria.'	Miller (1899).
<i>Houma</i>	'I have treated some two hundred cases, and have never seen a case but that had taken, in some form, a dose of quinine, while the system was suffering from a chronic malarial toxæmia.'	Menville (1901).
<i>General</i>	'Gives certain facts of his experience as regards malarial hematuria in a swampy, wooded region in Louisiana. . . . Quinine hemoglobinuria is also briefly mentioned. The parasite in the patient is the primary cause; quinine only acts as the provoking agent.'	Lerch (1901).
<i>Angola</i>	'The patient had lived in a malarial district for the past six months; since September, 1915, at Angola, La., and before that time, near Jackson, Miss.'	Ott (1916).
<i>Monroe</i> 1856	4 cases (1915-16) 'Discussion on the paper of Dr. Wright.' Dr. Leon T. Menville, Houma: 'According to the latest medical history, a doctor from Monroe, in 1856, was the first physician in the United States to report a case of black-water fever. . . .'	Wright (1917).

LOUISIANA—*continued.*

Locality and Date	Cases	Authority
<i>Le Roy</i>	Dr. J. T. Abshire, Le Roy: ' . . . We had plenty of it in my parish in years gone by.'	Thornhill (1921).
<i>Shreveport</i>	Dr. G. C. Chandler, Shreveport: ' During the first year of my practice I had an unlucky number of cases of blackwater fever, that is, thirteen.'	
<i>Arcadia</i>	' At Columbia, on the Ouachita river, where the first ten years of my professional experience was acquired, I saw a great deal of this disease in its most typical and malignant form.' Describes two cases: ' There seems to be a growing belief in some sections that there is a certain causal relation between quinine and hemoglobinuria. . . . On the Ouachita river . . . there was a deeply-rooted belief among the laity, that quinine would produce the disease; with some it amounted to a morbid fear and the knowledge that they were taking the drug during an attack, added greatly to the gravity of the prognosis. I have seen a number of cases myself, in which the attack developed soon after taking large quantities of quinine. . . . I have never seen a case of malarial hemoglobinuria in a person who did not give a history of previous attacks of malaria, or was profoundly under the influence of quinine at the time of the attack.' (Note: the paper was written in 1903.)	

MISSISSIPPI.

Locality and Date	Cases	Authority
<i>Holmes County</i>	' Twenty-six years here in a miasmatic region (the heart of the Yazoo Valley) . . . It is generally those that have had intermittent fevers for some time and neglected these that have or are most liable to have malarial haematuria and among the first symptoms are the incessant vomiting of bilious matter and the sudden discolouration of the skin, frequently in six hours after the onset of the attack, and the intense yellow muddy appearance of the eyes, while the urine is loaded with bile and hemorrhage soon appears, sometimes almost entirely blood and in large quantity.'	Thornton (1886).

MISSISSIPPI—continued.

Locality and Date	Cases	Authority
Trinity	'The mortality in my own cases, only six in number, amounted to fifty per cent.'	Hamilton (1891).
Natchez	'Now, as to the treatment of haematuria when found, I use ergot in half-drachm doses every three or four hours, and strychnine hypodermically in full doses. . . I have treated eight cases thus or about as indicated, with no deaths.'	Ballard (1899).
General	'The editor of the <i>Journal</i> can find any number of such cases in the Mississippi Valley, providing he has money to induce a subject to take a dose of quinine; they generally take arsenic.'	Anon (1899).
Benoit	'Malarial haemoglobinuria (I call it so to prevent confusion with haematuria) as it prevails in this section (the Mississippi Yazoo Delta) is always a grave disease, the mortality, as I have observed, falling but little below fifty per cent.'	Jones (1892).
General	'Malarial hematuria, as its name implies, prevails throughout the whole Southern country, especially in all localities abounding in lakes, swamps, stagnant water-courses, etc.'	Jones (1894).
Stovall	'I have observed more than fifty cases of this disease, and in 19 cases have made repeated blood examinations. In only 5 of these have parasites been present, estivo-autumnal in 4, and double tertian in 1. The malarial nature of the remaining 14 was demonstrated by the leucocytic variation and pigmentation characteristic of malaria.'	McElroy (1903).
Rosedale	1 (1901) 1 (1902)	Sutherland (1903).
Mississippi Valley	Deaderick and Thompson (1916) state that: 'Fifty cases observed by McElroy (1905) were distributed as follows: Two in the first year of residence, three in the second, six between the second and the fifth, twenty-three between the fifth and tenth, eleven between the tenth and twentieth, and five after twenty years.' p. 232.	Deaderick and Thompson (1916), McElroy (1905).
Clarksdale, Friar's Point, Hillhouse, Tutwiler	'From 1889 to 1895, we (Dr. E. H. Marten, of Clarksdale, and Dr. Barton, of Hillhouse) treated sixty-eight cases without quinine, and without a single death.' Dr. W. H. Harrison, of Tutwiler, Miss., in reply to a letter, writes, 'My experience is against it (quinine), good and strong. . . I am now actually afraid of it.'	Buck (1906).

MISSISSIPPI—continued.

Locality and Date	Cases	Authority
<i>Eaglebend</i>	'The writer was called down in Eaglebend, Miss., to see Roscoe Dunn (white), aged six years, who had a malarial chill at 2 p.m. of Aug. 23rd, 1907, turned yellow as gold within two hours, and had copious hemorrhages from his kidneys, with a temperature of 106½ degrees F. . . . The writer was debarred from giving quinine to prevent a recurrence of the malarial chill at 10 a.m. of August 24th, because the child's parents claimed that they had lost two children, while living at Alexandria, La., by the family physician then giving the children sulphate of quinine. . . .' Describes another case and states that 'Since that time the writer has had calls to cases of malarial hematuria. . . .' Discussion.	Kiger (1925).
<i>Delta</i>	Dr. W. H. Scudder (Maversville): 'I remember, 25 years ago, that in every medical meeting in this State there was a paper on hematuria, but now it seems to have gone out of style. I am from the Delta, and we see more of it there than you do in the other portions of the State, and of course we still regard it as an important disease. But I am glad to say it is not as prevalent in the Delta as it used to be. . . . In some parts of Texas they call it Black Water Fever because the urine is not red, but really is black.'	
<i>Lefflore County</i>	'During two seasons, 1925 and 1926, in Lefflore County, in the delta region, we have heard of no cases in this county.' 'The older physicians here (Greenwood) and in adjoining counties of the Mississippi delta, tell me that haematuria was abundant until about 1910, when it began to diminish with the marked diminution of malaria which took place about that time. All agree that cases are now rare, several of them have not had a case for years.'	Barber (1926).

MISSOURI.

Locality and Date	Cases	Authority
<i>St. Louis</i>	'McLean reports a case of malarial hematuria which he considers caused by the use of quinine in large doses.' (Whether originating in Missouri is not clear.) 'A case under observation now in the City hospital, convalescing from tropical malaria, exhibits not only an enormous amount of urobilin in the urine, but also, after precipitating the urobilin, a very large amount of hemoglobin, though the urine, when passed, had a normal colour.' (Interpretation doubtful.)	McLean (1899). Richter (1913).

NEW YORK.

Locality and Date	Cases	Authority
<i>Brooklyn</i>	Locality of the case not stated. (Nature of case appears to be doubtful.)	Geiss (1900).
<i>General</i>	'Case of malarial haematuria with some peculiar features.—Dr. Andrew H. Smith read the history of the case (not reported). . . . Dr. W. Gilman Thompson. . . . Certainly haematuria in malaria affections was rare in this part of the country. . . . Dr. A. Alexander Smith said that within three years he had seen a case of malarial haematuria. . . . he had come originally from the South, where malaria was prevalent.'	Medical (1897).

NORTH CAROLINA.

Locality and Date	Cases	Authority
<i>Edenton</i> 1855	'From 15 to 25 years ago I am reliably informed that blackwater fever was very prevalent in this section of North-eastern North Carolina, but has probably for the past ten years been altogether extinct in the immediate vicinity of Edenton, although I have learned of one death during the past year at a point about 60 miles distant. In this area, which is adjacent to the Roanoke river in Northampton County, the older physicians tell me that blackwater fever was unknown prior to 1855, in which year the disease made its appearance.'	Boyd (1926).
<i>Edenton</i>	'Hemorrhagic malarial fever.—Dr. W. A. B. Norcom . . . expressed his opinion that the disease did not, as was claimed by some, appear for the first time a few years ago, but that it had long been recognised . . . it either begins <i>de novo</i> or as the result of long or frequent attacks of intermittent fever. . . . It begins usually but not invariably with a chill of about two hours' duration, attended with intense internal heat . . . a severe nausea is experienced, which leads to vomiting, at first of food, then bile and then in bad cases, of blood—the latter sometimes resembling the black vomit. There is a sighing respiration, insomnia, great restlessness, anxiety and an almost unquenchable thirst. . . . The skin assumes a yellowish, even a bronze colour. . . . The urine may simply contain large quantities of degenerated red-corpuscles, or in severe cases, blood and albumen. . . . In severe forms he dies, either conscious and from asthenia, or unconscious from uraemia; heart clot and cholesterinemia may also cause death.' (p. 571.)	Norcom (1874).

NORTH CAROLINA—*continued.*

Locality and Date	Cases	Authority
<i>General</i>	'He had received a letter from a doctor in a town in North Carolina, asking if malarial haematuria was frequent in the vicinity of New York, and added that in his section it was very frequent, occurring in from fifteen to eighteen per cent. of all cases of malaria.'	Medical (1897).
<i>Kinston</i>	'... I have not considered malarial haematuria because it is unquestionably a different disease from hemoglobinuria and is very likely of an accidental origin (over-dosing with quinine under certain conditions, etc.), and liable to occur in almost any type of malaria, and doubtless sometimes complicates malarial hemoglobinuria.'	Parrott (1901).
<i>General</i>	He quotes Surgeon-General H. R. Carter, as stating that, 'In the absence of statistics, I can only say that there is much malaria in eastern North Carolina. . . . In days not long gone by, there was a large amount of extremely severe malaria in this section, not less than there was in the Canal Zone, and there is from reports not a little now, especially blackwater fever and malaria of the cerebral type.'	Trask (1916).

OHIO.

Locality and Date	Cases	Authority
<i>Cincinnati</i>	'A white adult male, aged 40, with frequent attacks of chills and fever for over a year previous to passage of blood in his urine, had taken quinine irregularly during that time, but had not taken any for several weeks previous to his hematuria. Blood had appeared constantly in his urine for two weeks previous to his coming under my observation, at times, he claims, almost pure blood being passed. . . . Urinalysis: colour light amber, and reaction sp. gr. 1.010; sediment reddish, amorphous and very abundant albumin present and in considerable quantities.' (Whether blackwater or not seems uncertain.)	Brown (1899).

PENNSYLVANIA.

Locality and Date	Cases	Authority
<i>General</i>	'Of the seven cases which I have noted during fifteen years, five originated in Pennsylvania.'	Tyson (1883) <i>a</i> .
<i>Philadelphia</i>	An analysis of 1,780 cases from the Pennsylvania hospital, the Episcopal hospital, and the Philadelphia hospital. There is no clear evidence of blackwater fever.	Anders (1895).

SOUTH CAROLINA.

Locality and Date	Cases	Authority
<i>Georgetown</i> 1868	'The disease has been familiar to the profession of Georgetown only in the past fifteen years. . . . The resemblance of this disease to yellow fever is certainly in some cases very striking, so much so, that it has been called "Swamp Yellow Fever," but the symptoms given above are sufficient to distinguish it.' 'Malarial hemoglobinuria or hemorrhagic fever as it is called, is the result of profound malarial intoxication. . . .' 'The fall months seem to furnish many cases, yet it may occur at any season of the year; I have seen it in the midst of winter.'	Bailey (1883). Sparkman (1901). Sparkman (1905).

TENNESSEE.

Locality and Date	Cases	Authority
<i>Memphis</i>	'By careful clinical study, by a large bedside experience assisted by competent pathologists and microscopists, we have learned that it is the product, the sum total of neglected malaria—a malarial toxæmia. . . . This jaundice is so pronounced that the late Dr. Warren Stone, of New Orleans, denominated hemoglobinuria as pseudo-yellow fever. . . . The Delta physicians, as well as the laity, have learned by experience that the administration of quinine with these conditions present, is fraught with danger frequently precipitating an attack. . . . Let me admonish you gentlemen . . . not to journey . . . to South Africa to investigate the blackwater fever, but come South to the swamps of Arkansas, Mississippi, and Louisiana, where we have malaria in abundance and in all of its forms.'	Jones (1900).

TENNESSEE—continued.

Locality and Date	Cases	Authority
<i>Memphis—continued</i>	<p>'I have seen thirteen consecutive recoveries under the "eliminative" treatment; no such record can be shown under the quinine therapy. (Three more cases and no deaths at time of revision of this proof.)' 2 cases (1900)?</p> <p>'Last summer, during my illness, there were admitted to St. Joseph's hospital, 21 cases of malarial hemoglobinuria and all were treated with hypodermic injections of quinine; two recovered. On the other hand, I have now an unbroken series of 21 cases without a death. The main point is, avoid cinchonising these patients.'</p> <p>'Quinine has no place in the therapy of malarial methemoglobinuria or hematuria as it is called. . . . A glance at some kidney sections in my possession will verify this statement. The uriniferous tubules are blocked with granular detritus, not blood-clots, as in the hemorrhagic cases.'</p> <p>'It must not be inferred that the so-called benign tertian infections are never associated with pernicious symptoms, as I have seen both comatose paroxysms and hemoglobinuria associated with these parasites.'</p>	<p>Krauss (1900).</p> <p>Cox (1900). Krauss (1903).</p> <p>Goltman (1904).</p> <p>McElroy (1904).</p>

TEXAS.

Locality and Date	Cases	Authority
<i>General</i> 1866	<p>Deaderick and Thompson (1916) state that, 'Dr. H. C. Ghent (1868), of Port Sullivan, Texas, in 1866, reported hemoglobinuric fever endemic in parts of Texas.'</p> <p>'Dr. Ghent, of Texas, had treated forty-seven cases, with five deaths.'</p> <p>'Dr. W. C. Stirling, of Weaver, Texas, says in the <i>Atlanta Med. and Surg. Journal</i>, April, 1889, that hemorrhagic malarial fever is quite common in Texas, on the creeks and rivers. . . . Drs. Louis and Lynch, of Carroll's Prairie, Texas, assert that quinine sometimes produces the hemorrhage. While admitting that they are old physicians and have treated a great many cases, the author does not agree with them.'</p>	<p>Deaderick and Thompson (1916). Ghent (1868).</p> <p>Cochrane (1885)<i>b</i>.</p> <p>Stirling (1889).</p>
<i>San Jacinto</i>	1 (1890)	Dock (1894).
<i>Tyler, St. Louis, South-western Railway Hospital</i>	1 (1894) 1 (1895)	Woldert (1895). Woldert (1896).

TEXAS—continued.

Locality and Date	Cases	Authority
<i>St. Louis, South-western Railway Hospital of Tyler</i>	'Since the year ending June 30, 1893, there were five cases of malarial hemoglobinuria admitted to the wards.'	Woldert (1898).
<i>Galveston</i>	'A case of pernicious malarial fever of the hematuric variety occurring in my service last summer.'	West (1904).
<i>Texas and Louisiana</i> ...	'Accordingly, 2,000 blanks for report of cases were mailed to the physicians of Southern and Eastern Texas and Louisiana, selecting localities where it was presumed the disease most prevailed. Of those requested, 81 replied; 40 of whom could not report cases, while 41 reported 173 cases, to which I added 29 cases of my own, making a total of 202 cases reported by 42 observers.'	Shropshire (1903).
<i>Toakum</i>		
<i>Tyler</i>	24 (1902-9)	Woldert (1912).
<i>Colorado and Brazos Bottoms</i>	'In this view of the action of sulphate of quinine, I am sustained by most if not all the physicians of my section of the country—viz., the Colorado and Brazos Bottoms (Texas). It would be difficult to induce one of them to administer quinine in malarial haematuria, even after the haemorrhage has been checked, for fear of reproducing it.'	Smith (1900).
<i>Columbus</i>	'Dr. John H. Bowers, of Columbus, Texas. . . . stated to me that in his long experience, extending considerably more than half-a-century, he had never seen a case of malarial haematuria in which sulphate of quinine had been administered but what the patient died, and that he did not recollect one in which it was withheld but that the patient recovered. The testimony of Dr. Gibson and Dr. Moore . . . of Richmond, Texas, was to the same effect, as also was that of the late Dr. Gerard Alexander, of Wharton, Texas.'	
<i>Richmond</i>		
<i>Wharton</i>		

VIRGINIA.

Locality and Date	Cases	Authority
<i>Richmond</i>	'Many competent observers believe that in this fever, especially in the graver cases a disintegration or solution of the blood discs takes place, whereby the haematin is set free in the circulation; it then finds an outlet through the kidneys. . . . Nitric acid failed to reveal its (bile) presence in the specimens (of urine) examined by him.' (p. 311.)	Joyes (1877).

VIRGINIA—continued.

Locality and Date	Cases	Authority
Norfolk	'The disease is known in this state as "yellow chills" or haemorrhagic malarial fever, in North Carolina as Roanoke yellow fever, and in Alabama, as the yellow disease, and is of especial interest on account of its recent origin, rapid results, and high rate of mortality. . . . The disease, on its first appearance in Roanoke Valley, was at first supposed to be yellow fever and when, afterward, it was discovered to have a separate and distinct individuality, the term Roanoke was prefixed, to distinguish it from the true yellow fever.' (How many cases observed not stated.)	Field (1899).

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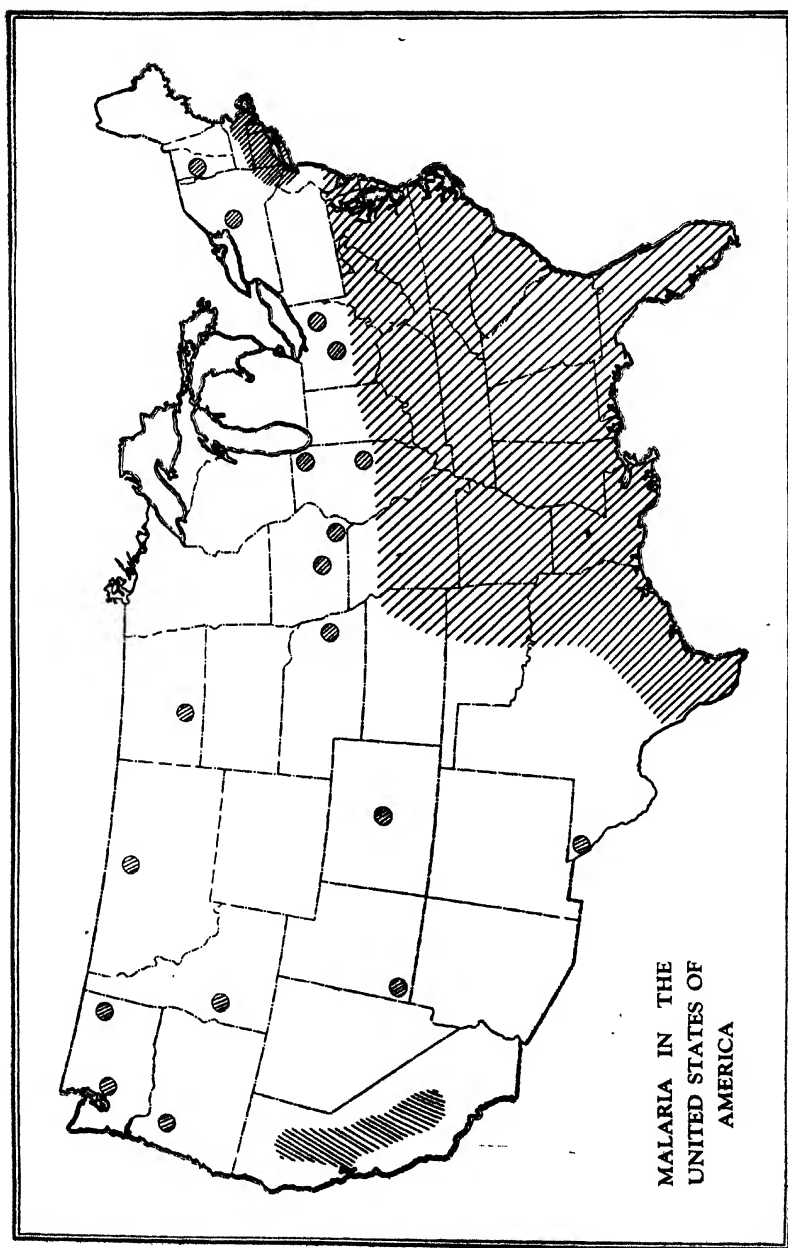
* Much of the literature on the subject has been unavailable. Many other references will be found in the 'Index Catalogue of the Library of the Surgeon-General's Office, U.S. Army,' Washington, and in the 'Index Medicus.'

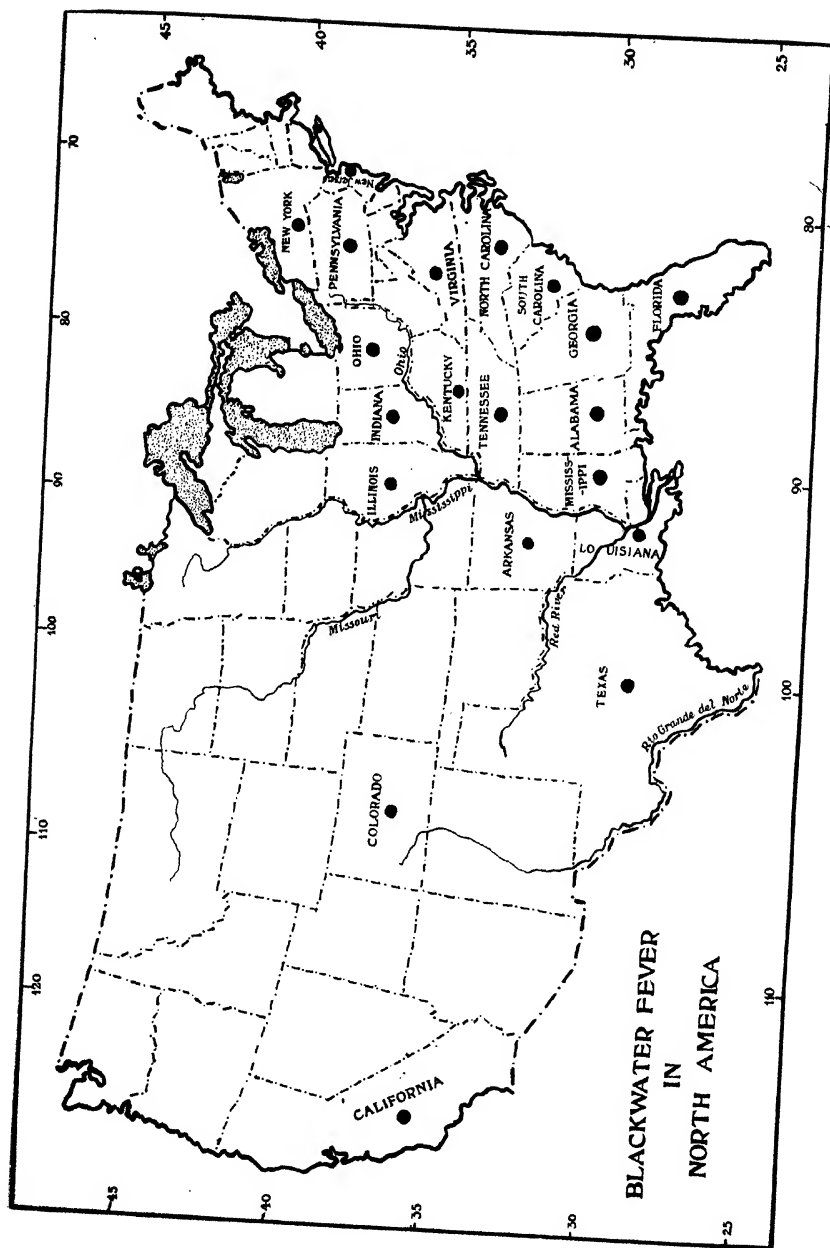
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ACANTHOCEPHALA FROM NORTHERN INDIA

I.—A NEW GENUS *ACANTHOSENTIS* FROM A CALCUTTA FISH

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PLATES X AND XI

Concerning the Acanthocephalous parasites of India very little is so far known, but lack of published records is no indication of scarcity of these parasites in our fauna. Within recent years some of them have been recorded and described from India and Burmah. Chandler (1925) was the first to bring to our notice a new species of *Centrorhynchus* from Calcutta. He was followed by Subramanian (1927 a, b, c), who dealt with a number of Burmese forms, and Thapar (1927) who created a new genus for a worm obtained by him from a Cyprinid fish at Lucknow. One of us (Datta, 1928) has also published an account of a new species, *Echinorhynchus robustus*, from the common crows of Allahabad.

In the course of our parasitic survey we have collected a number of interesting Acanthocephala from different groups of hosts, and the present paper is the first of a series dealing with them. The initial study was restricted to fish species and two preliminary abstracts were submitted to the Indian Science Congress held in Calcutta, in January, 1928. One of these forms the subject of this communication and the other is being prepared for the press.

ACANTHOSENTIS n.g.

Diagnosis. Acanthocephala of small size, parasitic as adults in the alimentary canals of fishes. Proboscis short, cylindrical to globular, armed with three circles, each composed of six single-rooted hooks. Receptacle of proboscis cylindrical, with a single

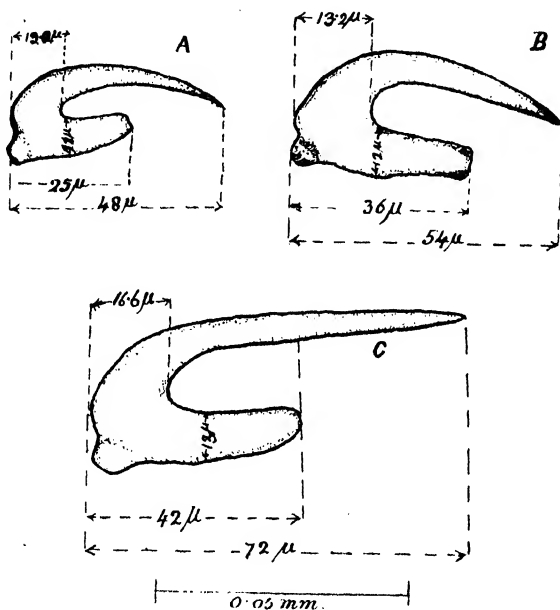
muscular wall. Body bearing 20 to 31 rings of close-set spines with a basal plate, on anterior two-fifths of its length; anterior circles crowded, posterior gradually becoming more widely separated. No spines behind middle of body. Retraction of proboscis followed or accompanied by drawing in of anterior body. Subcuticula with branched and oval nuclei. Central nerve ganglion near base of proboscis sheath. Lemnisci slender, cylindrical, longer than the proboscis receptacle. Genital organs of male in posterior half of body cavity; of female in posterior fourth or fifth. Testes ovoidal or ellipsoidal, contiguous, followed immediately by a rounded prostatic mass of syncytial nature, with from six to eight large nuclei. Ovary seen in very young specimens, as a small oval, near base of proboscis sheath. Uterus with six to eight short diverticula at commencement; vagina narrow with two glands. Embryos elongated, enveloped with three concentric membranes.

***ACANTHOSENTIS ANTSPINUS* n.sp.**

Description. The small siluroid fish *Aoria* (Macrones) *gulio* Günther (= *Pimelodus gulio* Ham. Buch), found in abundance in the fish markets of Calcutta from April to October and, in lesser numbers, throughout the year, was heavily infected with this worm. Of 90 to 100 fishes dissected during the months of May, June, and July, 1927, and of about half as many examined in October, the same year, nearly 80 per cent. harboured this parasite in the intestine. The number in each host varies from a few to nearly one hundred. They generally abound in duodenum and small intestine, but are also often met with in stomach and large intestine. As usual they are firmly attached to the intestinal wall; but in some cases they were observed floating freely in the lumen of the gut. On removal to salt solution, these Acanthocephalans gradually straighten out and perform slow movements by contraction and expansion of the body, but appear incapable of moving in a definite direction even for a short distance. Their internal anatomy can be studied in life under pressure of a slide as the cuticle and body-wall are fairly transparent. The specimens are best fixed expanded after having been left overnight in normal salt solution, in cold storage. Flattened unstained mounts show the details of the spines and the internal musculature better than stained preparations.

The colour of the worms is white, semi-transparent, but the larger females have a brownish appearance owing to large numbers of brown eggs within them.

The proboscis is short, cylindrical to globular, and is studded with strong recurved hooks of the shape and dimensions given in Text-fig. 1 and Table I. They are arranged in three circles of six hooks each. Each hook is provided with a stout undivided root



TEXT-FIG. 1. *Acanthosentis antispinus*, n.sp. Details of proboscis hooks: A.—Of basal circle; B.—Of middle circle; C.—Of terminal circle.

TABLE I.

Size of proboscis hooks in terms of μ

	Length of free portion	Breadth of free portion	Length of root	Breadth of root
Terminal circle	72.0	16.6	42.0	13.0
Middle circle	54.0	13.2	36.0	12.0
Basal circle	48.0	12.0	25.0	11.0

firmly implanted in the proboscis wall and bears an inconspicuous knob-like projection, at the anterior angle, near the commencement of the free portion.

The neck is completely wanting, although a faint depression marks the junction of the proboscis and the body proper.

The individuals vary in dimensions according to age, but average specimens, during life, measure in mm. as follows :—

	Length	Breadth
Males	1.0–1.25 mm.	0.25–0.2 mm.
Females	2.0–3.0 mm.	0.75–1.0 mm.

The males, as a rule, are more slender and smaller than the females of the same age and the largest of them, hardly exceed 2 mm. in length, whereas the largest females attain a length of 6 mm. or even slightly more. In Tables II and III are given relative measurements of male and female specimens, of different ages, respectively. The body is usually thickest about the middle, but in some individuals, in which the anterior end is drawn in, the broadest part is the anterior extremity [Pl. X, fig. 8 and Text-fig. 2, c.]. The anterior two-fifths of the body are covered with 20 to 31 circular rows of minute, pointed spines with flat scaly bases or platelets having an irregular margin [Pl. X, fig. 3] as in *Quadrigyryrus torquatus* (Ortlepp, 1924). Of these a few of the anterior and posterior circles have a smaller number of spines, but the middle ones carry 80 to 100 spines in each ring. In some of the older worms the spines towards the ventral surface, in the last three to six rows, appeared to have dropped. The genital pore is subterminal and lies in a depression directed postero-ventrally.

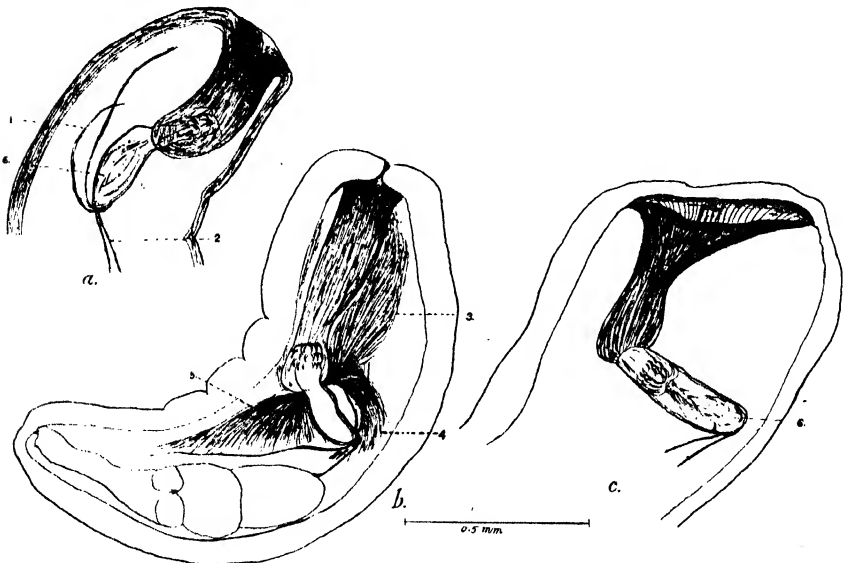
A thin cuticle forms the external covering of the body-wall. Underneath it lies the thick hypodermis or subcuticula. The latter is traversed by a system of narrow canals forming the lacunar system of transverse vessels (seen clearly only in living specimens or in preparations of the body-wall) irregularly anastomosing with one another and connected with a pair of indistinct longitudinal canals. A few large oval nuclei [Pl. X, fig. 5, o.n.] are clearly

discernible, in some preparations, situated laterally behind the proboscis sheath: they may be present elsewhere also [Pl. XI, fig. 12, *o.n.*]. There also occur in the subcuticula giant nuclei of the type described by Van Cleave (1921) in the genus *Quadrigyus*. In whole mounts they are easily seen in two or three places along the dorsal and ventral sides of the animal arranged either singly (anteriorly) or in groups of three or more (posteriorly) [Pl. XI, figs. 10, 12 and 13, *b.n.*]. On examining stained preparations of portions of body-wall similar but less prominent, and more branched, nuclei are met with in other places as well. We concur fully with Van Cleave (1928) in his interpretation of their nuclear nature, and agree with him that the doubt expressed by Baylis (1927) is unfounded. Internally to the hypodermis lies the narrow layer of longitudinal muscles followed by a layer of circular muscle fibres.

The retraction of the proboscis (with or without inversion) and of its sheath inwards into the body-cavity is often accompanied or followed by a drawing in of the anterior end of the worm. The in-drawn body-wall forms a characteristic tubular structure, with a funnel-like opening, hanging in the body-cavity. This peculiarity causes an interesting disposition of the muscles of the interior of the animal, which was studied by observing the movements of living acanthocephala under pressure of a glass slide. The drawing in of the front part is brought about by means of a double set of retractor muscles [Text-fig. 2, *b*, 4 and 5] which spread out posteriorly, in fan-shaped manner, dividing the body cavity into three portions—a dorsal, a ventral, and a median—separated from one another only incompletely [Pl. XI, fig. 15, *r.b.*]. Of the two sets of retractors the dorsal is more developed than the ventral and extends further posteriorly. The eversion of the drawn end is effected partly by the pressure exerted by the outer body-wall, partly by the relaxation of the retractors and partly by a protractor muscle [Text-fig. 2, *b*, 3].

The proboscis is hollow and retractile; it can be withdrawn into its sheath or may remain outside it in the tubular indrawn body-portion. At first it passes medially, without inversion; but in the fully retracted state, it gets inverted and is drawn, along with its receptacle, towards the dorsal side of the body-wall [Text-fig. 2, *c*]. The sheath of the proboscis has a single muscular wall with transverse muscle fibres strongly developed and is nearly

one and a half times longer than the proboscis. The invertors of the latter fill the interior of the sheath and are attached to its anterior tip [Pl. X, fig. 1, *rp.* and Text-fig. 2, *c*, 6]. In addition to these muscles confined within the receptacle, there pass out of its wall posteriorly two narrow but distinct retractors which run into the body-wall of the animal about the middle of its length. These draw the sheath into the interior of the body-cavity. The protrusion of the proboscis receptacle is the combined effect of the



TEXT-FIG. 2. Camera lucida sketches of pressed specimens showing various stages in the retraction of the proboscis, its sheath and body end; 1 and 2.—Protractors and retractors of proboscis sheath respectively; 3.—Protractor of body end drawn in; 4 and 5.—Its retractors; 6.—Invertor of proboscis.

relaxation of its retractors, the pressure of the body-wall of the worm and the contraction of two protractor muscles [Text-fig. 2, *a*, 1] which, arising from its hinder border, appear to run forwards on the side of the sheath opposite to that to which it is drawn in, namely towards the ventro-anterior end of the body.

The central nervous system consists of a single ganglion situated within the muscular layer of the proboscis sac near the posterior end of it. In some preparations two main trunks, the retinaculi, are clearly seen arising from the nerve cell. After passing out of the

sheath they take an undulating forward course before turning backwards and becoming gradually indistinct.

The lemnisci are as usual two and slightly longer than the proboscis sac. Each is provided with a single large nucleus placed posteriorly.

The male genitalia consists of a pair of testes, vasa efferentia, vas deferens, seminal vesicle, prostatic mass, penis, and bursa. The two ellipsoidal or ovoidal testes, which are nearly equal in size in extremely young males, are unequal in maturing as well as adult individuals (for relative measurements see Table II). They lie closely apposed to each other just behind the middle of the body in the median line. An efferent duct arises from each and runs backwards before joining its fellow to form the vas deferens, at the commencement of which lies the globular seminal vesicle. The vas deferens is a fair-sized tube full of sperms in mature males. In some mounted specimens and in longitudinal sections the anterior portion of the vas deferens appears saccular and packed full of sperms. After a short, more or less straight, course backwards it narrows and terminates in a small muscular cone, the penis. The prostatic gland consists of a rounded, syncitial mass containing six to eight nuclei. It lies in close proximity of the hinder testis, partly overlapping the seminal vesicle. It communicates with a wide tube, the prostatic duct, lying alongside the vas deferens and opening into the base of the penis by a narrow opening. The penis projects into the cavity of the bursa, which is a bell-shaped cuticular structure with a frilled margin and a pair of sacs. The whole genitalia is covered by a thin membrane of connective tissue and is connected through the genital ligament with the base of the proboscis receptacle.

The female genitalia consists of ovary, bell, uterus, ovejector, vagina, and vaginal glands. The ovary is visible as a tiny oval, just behind the proboscis sac, in preparations of very small females. At the termination of the post-larval stage it breaks up into packets of cells and hence cannot be seen in toto mounts of mature females. The ovarian packets of cells increase in number and size and, after rupturing the walls of the genital ligament, escape into the body-cavity in which they float about. Each of these egg masses is enveloped by a thin membrane and contains ova in various stages

TABLE II.
Measurements of flattened, preserved male specimens.

No. of specimen	¹ (large, adult)	² (medium)	³ (small, immature)
Total length of individual ...	mm. 2.4	mm. 1.7	mm. 0.8
Breadth—			
At anterior end ...	0.38	0.21	0.08
In middle of body (maximum) ...	0.67	0.34	0.18
At posterior end ...	0.29	0.17	0.08
Proboscis—			
Length ...	0.17	0.07	0.04
Breadth ...	0.05	0.04	0.04
Proboscis sheath—			
Length ...	0.38	0.25	0.17
Breadth ...	0.13	0.07	0.04
Lemnisci—			
Length ...	0.04 (straight)	0.34 (curved)	0.17 (contracted)
Breadth ...	0.07	0.05	0.04
Indrawn body end—			
Length ...	0.34	—	—
Breadth ...	0.08	—	—
Total length of genitalia ...	1.7	0.63 (excluding bursa)	0.38
Testes—			
Anterior, length ...	0.47	0.25	0.08
breadth ...	0.3	0.13	0.06
Posterior, length ...	0.34	0.17	0.06
breadth ...	0.32	0.15	0.05
Prostatic mass—			
Length ...	0.17	0.07	0.025
Breadth ...	0.21	0.08	0.042 (developing)
Seminal vesicle—			
Length ...	0.13	0.1	indistinct
Breadth ...	0.19	0.8	—
Bursa—			
Length ...	0.21	0.22	0.04 (indistinct)
Breadth ...	0.13	0.14	0.04

of development. When the ova are fully developed they break away from the egg mass and fill the entire body. The fertilisation is, as usual, internal and the embryo formation commences while the egg is still within the body of the mother. The fertilised eggs are characterised by the presence of three concentric enveloping membranes round the embryo which bears a tuft of spines towards the broader end. The eggs are elongated [Pl. X, fig. 9] when ready to be laid and measure 26μ by 8μ . They are brownish in colour.

The bell, or uterine bell as it is sometimes called, is a triangular funnel-like apparatus with a wide opening into the body-cavity. It is attached to the base of the proboscis sheath by means of the genital ligament [Pl. XI, fig. 10, *gl.*] which keeps it in position. At the posterior end of the bell, leading into the uterus, are given out a number of diverticula (six to eight) which allow only properly mature eggs to pass into the uterus. The less mature ones are returned into the body-cavity through a minute dorsal opening for further development. The uterus [Pl. XI, fig. 11, *ut.*] has thick flabby walls containing nucleated, flask-shaped glands. The uterus leads into the vagina through a muscular bulb, the ovejector, which appears to control the passage of eggs into the narrow vagina. The latter is long and the eggs generally pass through it in single file. The vagina terminates in the genital opening which lies at the base of a depression at the postero-ventral extremity of the worm. Outside the vaginal wall lie two single-nucleated, club-shaped glands which communicate with the vagina by a common pore close to its external aperture. They probably secrete a fluid which lubricates the genital opening.

Systematic position of ACANTHOSENTIS n.g.:—So far only eight genera of adult Acanthocephala from fishes are known, in which both the proboscis as well as the body surfaces are armed. The present genus, which differs from them all, as will appear from the comparative Table IV, is the ninth. The first four of the genera mentioned in the above Table, on account of their long proboscis with numerous hooks, double-walled proboscis sheath and several prostatic gland cells—not forming a single syncitial mass—stand apart from the last five which agree together in possessing a short proboscis with a small number of hooks arranged in three or four rows, single-walled proboscis receptacle and a syncitial prostatic

TABLE III.

Measurements of flattened, preserved female specimens.

No. of specimen	1 (large, mature)	2 (medium)	3 (small, immature)
Total length of individual ...	4.5 (proboscis everted)	3.5 (proboscis partly indrawn)	0.85 (proboscis inverted)
Breadth—			
At anterior end ...	0.6	0.9	0.2
In middle of body (maximum) ...	1.1	1.2	0.25
At posterior end ...	0.4	0.5	0.08
Proboscis—			
Length ...	0.25	0.15 (just near)	0.05 (in sheath)
Breadth ...	0.15	0.16	0.04
Proboscis sheath—			
Length ...	0.4 (near extremity)	0.6 (in middle line)	0.2 (lateral)
Breadth ...	0.18	0.15	0.06
Lemnisci—			
Length ...	0.9	twisted	0.14
Breadth ...	0.1	—	0.03
Indrawn body end—			
Length ...	—	0.7	0.15
Breadth ...	—	0.3	0.06
Total length of genitalia ...	0.9	0.7	0.25
Bell—			
Length ...	0.3	0.25	0.05
Breadth ...	0.25	0.2	0.04
Uterus—			
Length ...	0.25	0.28	0.03
Breadth ...	0.05	0.05	0.01
Vagina—			
Length ...	0.3	0.28	0.12
Breadth ...	0.05	0.05	0.01
Vaginal gland—			
Length ...	0.15	0.08 (contracted)	indistinct
Breadth ...	0.06	0.05	—

mass with a few large nuclei, except *Acanthogyrus* in which it is double with two or more nuclei in each. The new genus, included in the latter group, is readily distinguished from the others by the number and arrangement of its proboscis hooks, by the structure of the prostate, by the structure and disposition of the body spines and by the character of its subcuticular nuclei.

Southwell and Macfie (1925) placed the first three genera, namely, *Rhadinorhynchus*, *Telosentis*, and *Serrasentis* in the family Rhadinorhynchidae, sub-order Echinorhynchiea. For the fourth, *Tegorhynchus*, together with some others, they created a new family Corynosomidae under the same sub-order as Rhadinorhynchidae. Travassos (1926) has placed all the four together in the family Rhadinorhynchidae with two others: his system of classification does not erect orders and sub-orders, groups higher than the family. Thapar (1927) has taken a new line by disregarding the basis of previous classifications. He suggests the presence or absence of spines on the body and proboscis, and the single or double root of proboscis hooks as characters showing natural affinities. The first of the above two characters have been incorporated in the previous systems referred to above, though in a different way; but Thapar had not seen Travassos's (1926) publication as there is no reference to it in his paper. The other character is a new suggestion worthy of consideration by future workers on the group. In the tentative scheme of classification proposed by this author the first six genera of Table IV are inserted in his new family Acanthogyridae, which includes a number of other genera as well, all characterised by possessing single-rooted proboscis hooks. The family is placed in his new order Acanthogyridea (class Acanthocephala), all members of which have cuticular spines on the body. According to this criterion the last three genera also fall in the same category, though Van Cleave (1928), apparently unaware of Thapar's work, has erected a new family, Pallisentidae, for his two genera *Pallisentis* and *Neosentis*. Thus Thapar has massed together forms differing from one another in important details of anatomy such as the size of proboscis, number and arrangement of hooks on it, the character of the wall of its receptacle, the location of the central nervous system and the nature of the prostate gland.

It is not desirable on our part to suggest radical changes in the

existing systems (imperfect though they may appear) based on the direct study, however careful, of a few genera only. We therefore hope to revert to this subject later, after our more extensive survey of Indian types is completed, for it may throw illuminating light on the points at issue. With our present knowledge of the group, we consider it premature to give an isolated character (as the single or double root of hooks on the proboscis) predominance over a combination of no less important characteristics, in making it the basis of division into families. Moreover there exist types, like *Acanthocephalus anguillae* (Müll) [Lühe, 1911, page 14], in which the proboscis hooks have neither single nor double root, but are distinctly three-rooted (tri-radiate). In quite a number of other forms the roots show great variation in passing from one end or side of the proboscis to the other. A reptilian worm obtained by one of us (Verma), which will be described later, has the anterior hooks very complicated but the posterior ones extremely simple.

For the present, therefore, adopting Travassos's (1926) classification, we propose coupling our genus *Acanthosentis* with *Quadrigyrus*, with which it shows the closest affinity, into the sub-family Quadrigyrinae (Van Cleave, 1920) of the family Neoechinorhynchidae (Travassos, 1917). According also to Southwell and Macfie's scheme it will fall in with the same genus, but in the family Quadrigyridae of the sub-order Neoechinorhynchidae, order Acanthocephala. The family of Travassos is nearly equivalent to the sub-order of Southwell and Macfie, and his sub-family to the latter authors' family. Further, to avoid unnecessary multiplication of families for closely related forms, it would be better to include the genera *Acanthogyrus*, *Pallisentis* and *Neosentis* in the same family or sub-family with the above two genera, and to allow the families Acanthogyridae and Pallisentidae to merge into Quadrigyridae with the following emended definition :—

Acanthocephala of small to medium size. Proboscis short, with few rows of simple rooted hooks. Body armed with spines arranged in one or two series of complete or incomplete rings or scattered or both. Receptacle of proboscis with single muscular wall. Brain at or near base of receptacle. Prostate a syncitial mass, rarely double, with few large nuclei. Nuclei of subcuticula oval or branched or both. Parasitic in fishes.

TABLE IV.

Diagnostic characters of piscine genera of Acanthocephala, possessing armed proboscis and body.

Name of genus	Proboscis and its hooks	Wall of proboscis receptacle	Location of brain in receptacle	Prostate gland	Body spines	Subcuticular nuclei
<i>Rhadinorhynchus</i> ... Lühe, 1911	Long : hooks numerous, ventral stronger than dorsal.	Double.	Near middle	Not a single syncytial mass.	Scattered, powerful ; only on anterior part.	Small and numerous, or few large finely dendritic.
<i>Trilosentis</i> ... Van Cleave, 1920	do.	do.	do.	do.	Scattered ; on posterior extremity only.	
<i>Serrasentis</i> Van Cleave, 1923	do.	do.	do.	do.	A collar anteriorly, followed by 18 to 23 ventral rows.	
<i>Tegorhynchus</i> ... Van Cleave, 1920	Long : hooks numerous, but symmetrical.	do.	At anterior end.	do. (several cells, elongated)	An uninterrupted mantle from anterior end backwards ; none on posterior end.	
<i>Onadrigyrus</i> ... Van Cleave, 1921	Short : 4 circles of 5 each.	Single.	Near base.	Compact, syncytial ; nuclei few, large.	In four circles ; on anterior surface.	Two ovoid, anterior ; few branched scattered.
<i>Acanthogyrus</i> ... Thapar, 1927	Short : 3 circles of 8 each.	do.	At base.	Spherical double mass ; nuclei two or more in each.	19-20 circles anteriorly followed by 20-21 paired lateral ones, to posterior end.	—
<i>Pallisentis</i> ... Van Cleave, 1928	Short : 4 circles of 6 each.	do.	Near base.	Very long, syncytial ; nuclei few, large.	6-9 rings anteriorly ; 20-40 rings further back.	—
<i>Neosentis</i> ... Van Cleave, 1928	Short : 4 circles of 8 each.	do.	At posterior end.	Long, syncytial ; nuclei 16, large.	5-6 circles anteriorly ; 6-8 further back ; scattered ones on anterior third.	—
<i>Acanthosentis</i> ... n.g., 1928	Short : 3 circles of 6 each.	do.	Near base.	Compact, syncytial ; nuclei 6-8, large.	20-31 rings on anterior two-fifths.	Few oval and branches.

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PLATE X

EXPLANATION OF PLATE X

- Fig. 1. Lateral view of male specimen pressed and mounted.
 Fig. 2. Proboscis showing arrangement and number of hooks.
 Fig. 3. Some body spines, highly magnified.
 Fig. 4. Photomicrograph of transverse section through anterior region of a male worm.
 Fig. 5. Young male with developing prostate.
 Fig. 6. Male genitalia, after dissection.
 Fig. 7. Vertical section of adult male passing through the reproductive organs.
 Fig. 8. Magnified view of three eggs.

All figures, with the exception of Number 4, were drawn with Abbe's Camera Lucida.

REFERENCES TO LETTERING

<i>b.</i>	= bursa.	<i>p.</i>	= proboscis.
<i>b.n.</i>	= branched nucleus.	<i>p.d.</i>	= prostatic duct.
<i>b.s.</i>	= body spines.	<i>pe.</i>	= penis.
<i>c.</i>	= cuticle.	<i>p.g.</i>	= prostatic gland or mass.
<i>c.m.</i>	= circular muscle fibres.	<i>p.h.</i>	= proboscis hooks.
<i>d.r.p.s.</i>	= dorsal retractor of proboscis sheath.	<i>p.s.</i>	= proboscis sheath or receptacle.
<i>e.m.</i>	= egg mass.	<i>r.b.</i>	= retractor muscle of body end.
<i>g.l.</i>	= genital ligament.	<i>r.p.</i>	= retractor or inverter muscle of proboscis.
<i>g.p.</i>	= genital pore.	<i>s.m.</i>	= spongy muscles.
<i>h.</i>	= hypodermis or subcuticula.	<i>s.v.</i>	= seminal vesicle.
<i>l.</i>	= lemnisci.	<i>t.</i>	= testes.
<i>l.m.</i>	= longitudinal muscle fibres.	<i>u.</i>	= uterus.
<i>n.</i>	= nucleus.	<i>u.b.</i>	= uterine bell.
<i>n.f.</i>	= nerve fibre or retinaculum.	<i>u.d.</i>	= uterine diverticula.
<i>n.g.</i>	= nerve ganglion.	<i>v.</i>	= vagina.
<i>o.</i>	= ovary.	<i>v.d.</i>	= vas deferens.
<i>oe.</i>	= ovejector.	<i>v.c.</i>	= vas efferens.
<i>o.m.</i>	= ova with enveloping membranes.	<i>v.g.</i>	= vaginal gland.
<i>o.n.</i>	= oval nucleus.	<i>v.r.p.s.</i>	= ventral retractor of proboscis sheath.
<i>ov.</i>	= eggs or fertilised ova.		

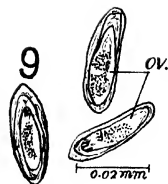
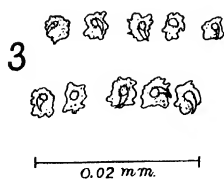
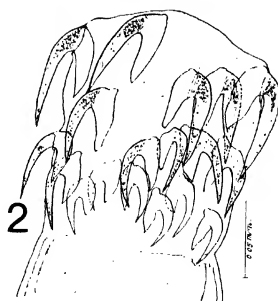
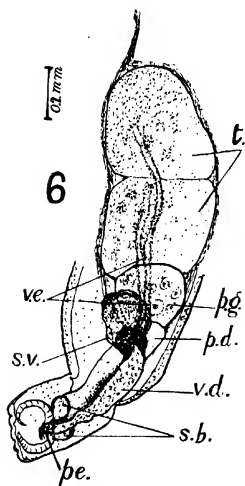
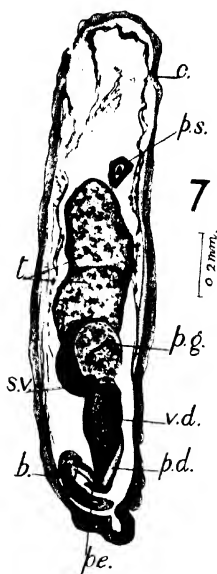
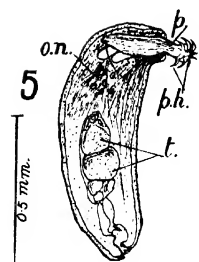
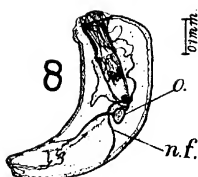
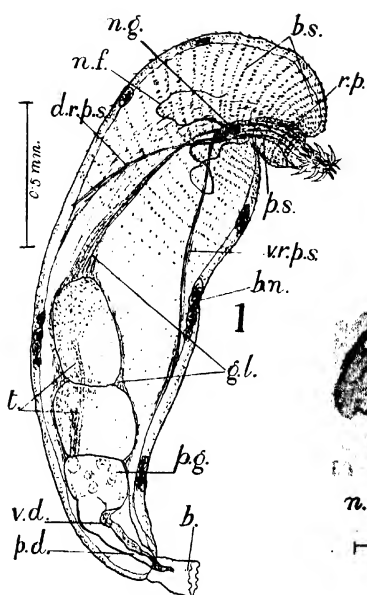


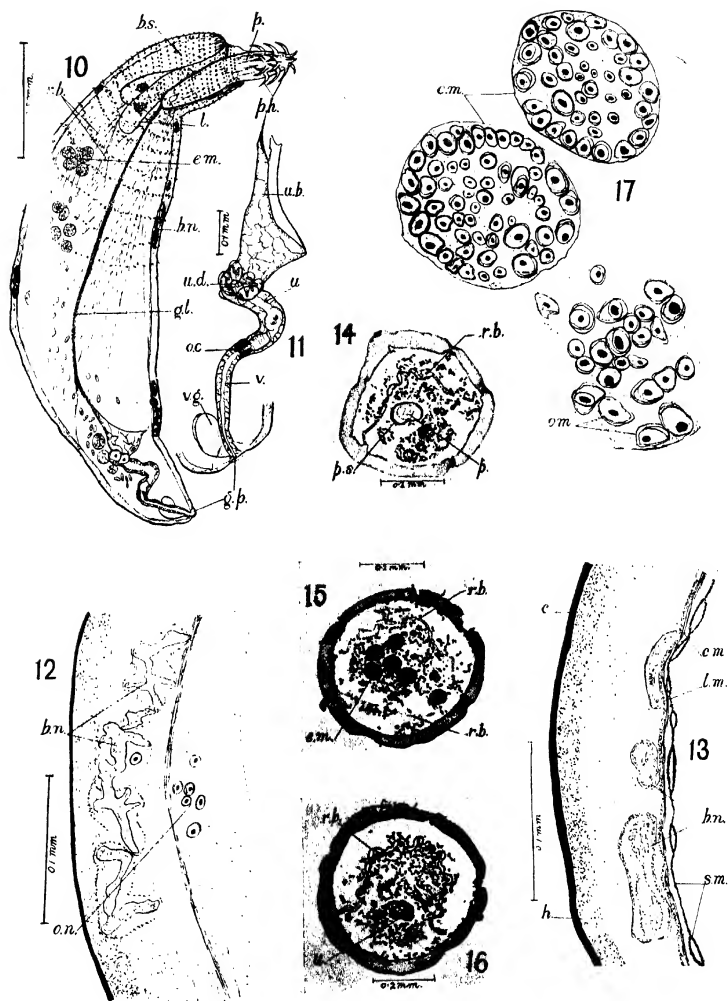
PLATE XI

EXPLANATION OF PLATE XI

- Fig. 10. Lateral view of adult female, pressed and mounted.
- Fig. 11. Female genitalia after dissection ; much magnified.
- Fig. 12. Part of body-wall in toto mount showing branched and oval nuclei.
- Fig. 13. Transverse section of body-wall through a branched nucleus.
- Figs. 14-16. Photomicrographs of transverse sections of a female specimen passing through the proboscis sheath, the middle of the body, and the uterus respectively.
- Fig. 17. Egg masses, entire and split up, showing developed ova.

All figures, except Numbers 14 to 16, were drawn with Abbe's Camera Lucida.

For references to lettering see explanation of Plate X.



STUDIES IN CHEMOTHERAPY*

I. A METHOD FOR MAINTAINING PATHOGENIC TRYPANOSOMES ALIVE *IN VITRO* AT 37° C. FOR 24 HOURS

BY

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AND

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As a preliminary step in an investigation designed with the object of obtaining some insight into the mechanism of the action of certain arsenical preparations in experimental trypanosomiasis, it appeared desirable to examine the action of the drugs in question on the parasites *in vitro*.

Unfortunately, all attempts to culture the pathogenic trypanosomes have hitherto been unsuccessful, and yet it is at once obvious that before we can proceed with an investigation of this nature, some method must be discovered whereby the trypanosomes can be maintained alive *in vitro* for such a length of time as will enable one to judge definitely whether the addition of the drugs to the medium has, or has not, any trypanocidal effect. During the past thirty years many workers have interested themselves in the question of the action of drugs on trypanosomes *in vitro*, and the number of papers relating to the subject is very considerable. It is, consequently, not surprising that the literature dealing with the maintenance of trypanosomes *in vitro* is also voluminous. We do not propose to refer to the great majority of these papers, beyond observing that they reveal many contradictory statements and

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anomalies and, generally speaking, are of little value for the object we have in view, as they contain no precise quantitative information. There seems to be, however, a more or less general agreement that a medium consisting wholly, or partly, of serum maintains trypanosomes better than physiological saline and the various modifications of Ringer's solution; and that the parasites survive longer at laboratory temperature than at 37° C. Terry (1911 and 1915), who examined the trypanocidal action of certain drugs *in vitro*, concluded that all the sera he used—rabbit, ox, horse, goat, sheep, pig, chicken, rat and mouse—preserved the motility and the morphology of *Trypanosoma brucei* better and longer than did salt solution and various modifications of Ringer's solution. He states that the serum should not be diluted more than two to four times, that undiluted serum is the best, and that the parasites are better preserved at room temperature than in the ice-box; he adds that the infectivity of trypanosomes suspended in ox serum at room temperature was maintained for at least eight days. Apparently, no observations were made at 37° C. Rothermundt and Dale (1912), in an interesting paper on the action of atoxyl *in vitro* and in the animal body, likewise discuss the problem of maintaining trypanosomes *in vitro*. They found that whereas trypanosomes suspended in physiological saline died within a period of two hours at 37° C., and lived for only two to two and a half hours at laboratory temperature (22° C.), their survival was prolonged to a period varying from two to five hours at 37° C., and from eight to twenty-four hours at 22° C., when the parasites were suspended in defibrinated guinea-pig blood. They subsequently discovered that guinea-pig serum was distinctly superior to defibrinated blood, and that in this medium the parasites remained alive at 37° C. for at least five hours. Kligler and Weitzman (1925) studied the action of Bayer 205 *in vitro*, and during their work found that *T. evansi* could be kept alive in guinea-pig serum diluted with saline for at least twenty-four hours at 25° C.; no details are given beyond the fact that certain of the parasites were, at the end of this period, actively motile and infective; apparently no observations were made at 37° C. Papamarku (1927) made an extensive study of the action of certain drugs on trypanosomes and spirochaetes *in vitro*. He employed deactivated rabbit serum as a medium for suspending

the parasites ; this was inoculated by the addition of a drop of infected blood, and the medium was then covered with a layer of liquid paraffin. Under these conditions, Papamarku found that the motility of the trypanosomes was preserved for at least forty-eight hours at room temperature ; here again, however, there is no record of observations made at 37° C.

Preliminary experiments soon convinced us of the truth of the main conclusions reached by previous workers ; namely, that when suspended in serum the trypanosomes survive much longer than in physiological saline or in various modifications of Ringer's solution, and that the period of survival is considerably greater at laboratory temperatures than at 37° C. We were, however, early impressed by a fact which, although it had possibly not escaped the notice of those who had previously investigated the subject, yet is certainly not referred to by them. In many of our experiments a progressive diminution in the number of trypanosomes set in shortly after the commencement of the experiment ; careful microscopic examination showed that whilst many of the parasites were actively motile and of normal appearance, others were sluggish or even motionless, and some were clearly in various stages of disintegration. This, of course, means that under the same conditions of experiment certain individuals died relatively quickly and then disintegrated and finally became unrecognisable, whilst others remained actively motile and apparently in good condition for much longer periods. It should further be mentioned that in our preliminary work the results obtained were far from constant ; for example, in some experiments the trypanosomes suspended in rabbit serum died much more quickly than was the case in other experiments in which the same medium was employed.

Considerations of this nature led us, therefore, to re-investigate the whole subject, with the object of obtaining, so far as was possible, precise information having a quantitative value, and of devising a method whereby trypanosomes could be kept alive, without serious diminution in the original number introduced into the medium, for a period of at least twenty-four hours at 37° C. Notwithstanding the fact that it is generally recognised that it is much easier to keep trypanosomes alive *in vitro* at laboratory temperature than at 37° C., the ultimate object we had in view—the investigation of the

trypanocidal action of drugs *in vivo*—compelled us to concentrate our attention on experiments conducted at 37° C. It is a remarkable fact that almost without exception all those who have concerned themselves with the action of drugs on trypanosomes *in vitro* have worked at comparatively low temperatures. This can only mean a general recognition of the difficulty of keeping the parasites alive *in vitro* at body temperature.

As preliminary observations appeared to confirm the conclusion of previous workers, that serum was the best medium for supporting trypanosomes *in vitro*, we decided at first to confine our attention to this medium, and then, having ascertained under what conditions the best results were to be obtained with it, to compare those given with other media under similar conditions.

The trypanosomes used in these experiments were: (i) a strain of *T. equiperdum*, about which little is known beyond that it has been maintained for many years in European laboratories by passage through mice; (ii) a strain of *T. rhodesiense*, which was isolated from man in 1923, and which has since been kept in mice; and (iii) a strain of *T. gambiense* isolated from man in March, 1922, and since maintained in mice.

Technique. A mouse at the height of the infection, when its blood was swarming with parasites, was killed with chloroform, and blood obtained from the heart with aseptic precautions was diluted with twice its volume of sterile citrated saline solution. After mixing thoroughly, 1 volume of this was added to 9 volumes of rabbit serum; this, which we call Suspension A, was, therefore, a 30-fold dilution of the infected blood. Suspension B was made by taking 1 volume of Suspension A and adding it to 9 volumes of rabbit serum, and Suspension C by diluting 1 volume of Suspension B with 9 volumes of rabbit serum. By this means various dilutions of the infected blood in rabbit serum were obtained, viz., Suspension A, in which the infected blood was diluted 30-fold; Suspension B, in which the dilution was 300-fold; and Suspension C, in which it was 3,000-fold. About 1.0 c.c. of each of these suspensions was then added to each of a series of sterile glass tubes, and incubated at 37° C. These tubes, which were about 7.5 mm. in bore and 7.5 cm. in length, were made in the laboratory from ordinary stout-walled glass tubing, and were covered by caps made from glass tubing of a

slightly larger diameter.* Samples were removed from time to time with a sterile pipette—care being taken to mix thoroughly the contents of the tube—and examined, microscopically, on a Thoma Zeiss haemocytometer slide with the combination, Leitz Oc. 2, Obj. 6. By this means, it was found that the number of trypanosomes present in a suspension could be determined with reasonable accuracy, provided the concentration did not exceed about 60 parasites to the 256 small squares of the haemocytometer scale, i.e., approximately 1,000 per c.mm. It was found that in the case of very heavily infected mice, Suspension C, representing a 3,000-fold dilution of the infected blood, gave, as a rule, a count of between 30 and 60 parasites for the 256 squares of the haemocytometer scale.

TABLE I.

Tube	Concentration of infected mouse blood in fresh rabbit serum	Number of living trypanosomes per 256 squares of the haemocytometer scale							
		Start	2 hours	4 hours	6 hours	9 hours	11 hours	13 hours	25 hours
1	1 : 30	(7,000)	+++*	...	57*
2	1 : 30	+++*
3	1 : 300	(700)	...	57°	...	64°	...	62°	15*
4	1 : 300	56°	...	648	...	20*
5	1 : 3,000	67	82	...	108	88	79
6	1 : 3,000	105	84	...	66
7	1 : 30,000	7	7	16	19	9	...	14	5
8	1 : 30,000	10	...	13	14	6

The figures in brackets are calculated numbers, and the large numbers (over 100), have only an approximate value.

+++ Indicates living trypanosomes too numerous to count; many large agglomerations seen.

* Large numbers of dead and degenerate parasites also seen.

Numerous experiments of this nature were performed with the various strains of trypanosomes, using fresh and deactivated rabbit serum as a medium. The results obtained were constant and are illustrated by the typical protocol set forth in Table I.

* The discovery of a suitable covering for the tubes proved to be a matter of some difficulty. At first, following Papamarku, the contents of the tubes were covered with a layer of liquid paraffin; this was, however, found unsuitable for our purpose, as the paraffin droplets interfered with the enumeration of the parasites in the haemocytometer. Plasticine caps and cotton-wool plugs were also tried, but both had to be abandoned, as the heating of the tops of the tubes for the purpose of sterilization resulted in the production of substances toxic to the trypanosomes. The small amount of dust falling from sterilized cotton-wool plugs also seemed to have a detrimental effect on the trypanosomes, which showed a marked tendency to adhere to minute solid particles.

From the experiment recorded in this table it will be seen that when the infected mouse blood was diluted 3,000 times, the number of living parasites was substantially the same at the end of 24 hours as it was at the beginning of the experiment. This does not imply that all the individual trypanosomes originally present in the suspension had survived during this period. As a matter of fact, occasional dead and degenerate forms were seen throughout the whole period. The numbers were, however, maintained by multiplication of the parasites.

Divisional forms were seen throughout the experiment, and during the early hours division proceeded at such a rate that it more than balanced the loss due to death, with the result that the total number of parasites increased substantially. As time went on, the rate of multiplication either subsided, or the death rate increased, so that the total number of parasites gradually decreased until, at the end of 24 hours, the number was about the same as at the beginning of the experiment. This likewise applied in the cases where the dilution of the infected blood was 1 : 30,000. When, however, we turn to the observations where the dilutions were only 1 : 300 or 1 : 30, death occurred much more rapidly. In the 1 : 300 dilutions, the number of parasites remained more or less stationary for about 12 hours and then fell rapidly, so that after 24 hours only a small fraction of the original number was still alive. In the 1 : 30 dilutions death occurred with great rapidity; within an hour or so large agglomerations of sluggishly-moving parasites, many of which were degenerating, were to be seen, and within 6 hours the great majority were dead and degenerate.

As frequent observations of this nature indicated that the degree of dilution of the infected blood influences to a very definite extent the length of survival of the parasites in rabbit serum at 37° C., experiments were devised with the object of determining whether it was the concentration of parasites, or that of the serum of the infected mouse, which was the determining factor in producing this result.

EXPERIMENT. To 0.5 c.c. of citrated-saline was added 0.25 c.c. of the heart blood of a mouse, taken at the height of infection with *T. rhodesiense*, and from this, 30-fold (Suspension A), 300-fold (Suspension B) and 3,000-fold (Suspension C) dilutions in fresh rabbit serum were made as previously described. About 2.5 c.c. of Suspension A was centrifuged at high speed; the deposit of trypanosomes washed

thrice in fresh rabbit serum, and finally fresh rabbit serum added to the washed deposit so as to bring the volume once more up to 2.5 c.c., thus giving a concentration of washed parasites (Suspension D) corresponding to that of the 30-fold dilution of mouse blood. From this Suspension E and Suspension F were made, corresponding to the 300-fold and the 3,000-fold dilutions of mouse blood. Each of the six suspensions was divided amongst a number of tubes which were placed in a water bath at 37° C., and to one tube of each of these was added one-twentieth of its volume of the centrifuged serum of the infected mouse, with a view to ascertaining whether possibly any antibodies it might contain exercised an unfavourable action on the longevity of the trypanosomes.

The results of this experiment, which are set forth in Table II, indicate clearly that the factor which determines the longevity of the parasites in rabbit serum is not the concentration of the serum of the infected mouse, but that of the parasites. That this is so is not surprising, when one considers the enormous activity of the trypanosomes. Very little is known of the metabolism of trypanosomes. Experiments performed many years ago (Nauss and Yorke, 1911) showed that the incubation, in the absence of air, of living trypanosomes in defibrinated blood causes, if the parasites be numerous, a total disappearance of the oxygen combined with the haemoglobin; the carbon dioxide is not increased in a degree corresponding to the diminution of oxygen. There seems no reason to doubt that the parasites absorb their protein and carbohydrate material in the liquid state from the medium in which they are living; and it is quite probable that the shorter life of the parasites in the more concentrated suspensions is due, in part at least, to the more rapid exhaustion of the nutrient material in the medium. It is also possible that the products of metabolism are unfavourable to the life of the parasite, and that their more rapid accumulation in the concentrated suspension of trypanosomes constitutes another of the factors which curtail the length of life of the parasites in such suspensions, as compared with weaker suspensions.

The work of Biot, Biot and Richard (1911), and that of Schern (1925), and others, has shown the importance of glucose in the metabolism of trypanosomes. In our preliminary experiments we were impressed by the fact that serum which had been kept for some time, and which had become contaminated by bacteria, had lost, to a considerable extent, its power of supporting trypanosomes, even though it had been sterilized by heating to 60° C. for half an hour on several occasions before use. The addition of a small quantity

TABLE II.

Tube		Concentration of infected blood, or equivalent concentrations of washed parasites, in fresh rabbit serum	Number of living trypanosomes per 256 squares of the haemocytometer scale									
			Start	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours	10 hours	12 hours
1	Suspension A	1 : 30	(4,000)	+++	+++	...	+++	30*	10*	2*
2	Suspension A	(4,000)	50*
3	Suspension A + $\frac{1}{20}$ vol. of serum of infected mouse	(4,000)	+++	+++	...	+++	45*	13*	1*
4	Suspension B	1 : 300	(400)	450	440	435	485	435
5	Suspension B	(400)
6	Suspension B + $\frac{1}{20}$ vol. of serum of infected mouse	(400)	336	...	475	410	340	280	250
7	Suspension C	1 : 3,000	36	48	...	48	...	55	56	52
8	Suspension C	34
9	Suspension C + $\frac{1}{20}$ vol. of serum of infected mouse	46	47	...	47	...	56	57	46
10	Suspension D	1 : 30	(4,000)	+++	+++	++*	...	50*	10*	1*
11	Suspension D	(4,000)	45*
12	Suspension D + $\frac{1}{20}$ vol. of serum of infected mouse	(4,000)	...	+++	...	+++	++	5*	0*
13	Suspension E	1 : 300	(400)	380	...	392	...	380	352	358
14	Suspension E	(400)	392
15	Suspension E + $\frac{1}{20}$ vol. of serum of infected mouse	(400)	450	...	345	...	450	416	392
16	Suspension F	1 : 3,000	40	39	...	41	...	64	50	45
17	Suspension F	39
18	Suspension F + $\frac{1}{20}$ vol. of serum of infected mouse	34	40	...	58	...	52	46	53

The figures in brackets are calculated numbers, and the large numbers (over 100), have only an approximate value.
 +++ Indicates living trypanosomes too numerous to count; many large agglomerations seen.

* Large numbers of dead and degenerate parasites also seen.

(0.1 per cent.) of glucose to such a serum sufficed to restore in a large measure its nutrient qualities. With the object of investigating the matter further, experiments of the following nature were undertaken.

EXPERIMENT. Blood from the heart of a mouse at the height of infection with *T. rhodesiense* was diluted with an equal volume of citrated saline, and 1 c.c. of the citrated blood was then added to 9 c.c. of deactivated sheep serum. This mixture was placed in the incubator at 37° C. and shaken from time to time. After 3 hours it was centrifuged at high speed and the supernatant fluid, 'Extracted serum,' removed. The capacity to sustain trypanosomes of the 'Extracted serum,' and of the various modifications of it shown in Table III, was examined in the manner already described.

The data supplied in Table III demonstrate that the presence of large numbers of trypanosomes in serum for a period of 3 hours at 37° C. impairs, to a marked extent, its capacity to support trypanosomes, and furthermore, that the addition of 0.1 per cent. glucose suffices to restore its nutrient qualities. The conditions of the experiment failed to reveal the presence in the 'Extracted serum' of any substance highly toxic to the trypanosomes. If such toxic substances had been produced in any quantity, one would have

TABLE III.

Tube		Trypanosomes per 256 squares of the haemocytometer scale				
		Start	1½ hours	4½ hours	7½ hours	19 hours
1	Extracted serum	76	88	94	3	0
2	Extracted serum	1	0
3	Extracted serum + 0.1 % glucose	72	78	108	106	79
4	Extracted serum + 0.1 % glucose	95
5	Extracted serum 0.5 c.c. + normal sheep serum 0.5 c.c.	77	...	81	85	69
6	Extracted serum 0.5 c.c. + normal sheep serum 0.5 c.c.	...	77	60
7	Saline 0.5 c.c. + normal sheep serum 0.5 c.c.	71	...	74	77	71
8	Saline 0.5 c.c. + normal sheep serum 0.5 c.c.	...	62	64
9	Normal sheep serum	65	...	73	87	65
10	Normal sheep serum	73	65

expected that the mixture, consisting of equal parts of the 'Extracted serum' plus normal serum, would have proved less favourable as a medium for supporting trypanosomes than equal parts of physiological saline plus normal serum; such, however, was not the case.

In view of the striking results given by experiments of this kind, we decided to ascertain whether, by chemical analysis, it was possible to demonstrate that the suspension, in serum, of numerous trypanosomes at 37° C. for various periods, resulted in a removal of glucose from the serum.

EXPERIMENT. In each of two wide glass tubes, A and B, was placed 5 c.c. of sheep serum which had been heated to 56° C. for 30 minutes. To one of these, Tube B, was added 10 mgm. of glucose. The heart blood of a mouse heavily infected with *T. rhodesiense* was then withdrawn into an equal volume of citrated saline, and 0.5 c.c. of the citrated blood was added to each of the two portions of sheep serum. The suspensions were immediately placed in the water bath at 37° C. and agitated at frequent intervals. It was observed that the colour of each changed within a few minutes from bright red to dark purple, owing to the reduction of the oxyhaemoglobin of the mouse red cells. On shaking, the bright red colour was immediately restored.

At the end of an hour, Suspension A no longer turned purple, and examination showed that practically all the trypanosomes were dead; it was then centrifuged at high speed and the supernatant fluid 'Extracted serum A,' removed and set aside for sugar estimation. Suspension B continued to turn purple and the trypanosomes to exhibit active motility for a further period of 4½ hours, when both the reduction of the haemoglobin and the motility of the parasites ceased; it was then centrifuged and the supernatant fluid, 'Extracted serum B,' removed.

The sugar content of the original sheep serum and of the 'Extracted sera A and B' was determined with the following results:—

Original sheep serum, 50 mgm. per 100 c.c.

'Extracted serum A,' less than 10 mgm. per 100 c.c.

'Extracted serum B,' less than 10 mgm. per 100 c.c.

Analysis of the data supplied by this experiment conveys some idea of the enormous consumption of sugar in the metabolism of trypanosomes. The trypanosome suspensions consisted roughly of 5 c.c. of serum and 0.25 c.c. of infected mouse blood, i.e., the dilution of infected blood was approximately 20-fold, and the concentration of trypanosomes approximately 80,000 per c.mm. The quantity of sugar in Suspension A was 2.5 mgm. and that in Suspension B, 12.5 mgm. Apparently, therefore, the trypanosomes in 0.25 c.c. of infected mouse blood (i.e., 400 millions) sufficed, within 1 hour, to cause the disappearance of between 2 mgm. and 2.5 mgm. of sugar, and, within 5 hours, of between 12 mgm. and 12.5 mgm. In experiments of this sort, where concentrated suspensions of trypanosomes

were used, the length of life of the parasites appeared—within, of course, certain limits—to vary directly with the amount of sugar available.

Having ascertained that trypanosomes consumed large quantities of sugar, and that the length of life of the parasites in concentrated suspensions is largely determined by the amount of sugar available, we proceeded to enquire whether any evidence could be obtained that more prolonged sojourn of numerous trypanosomes in serum resulted in the production of changes in the serum other than the mere removal of glucose.

EXPERIMENT. To 15 c.c. of sheep serum was added 1.5 c.c. of a mixture of equal parts of citrated saline and the heart blood of a mouse heavily infected with *T. rhodesiense*, and the resulting suspension was immediately placed in the water-bath at 37° C. Within a few minutes the colour was observed to have changed from bright red to dark purple; on shaking, the red colour immediately returned. The suspension was shaken every few minutes during a period of 2½ hours, at the end of which time very little reduction occurred, and practically all the trypanosomes were found to be dead and degenerate; it was now centrifuged at high speed and the supernatant fluid removed and divided into 3 equal volumes, the first of which, 'Extracted fluid A,' was set aside; and to the third, C, was added sufficient glucose to produce a 0.1 per cent. concentration. To each of the Portions, B and C, was then added 0.5 c.c. of the citrated heart blood of another mouse heavily infected with *T. rhodesiense*, and the suspensions again placed in the water bath at 37° C. It was observed that both suspensions speedily became dark purple; they were frequently shaken as before. After 30 minutes it was found that Suspension B turned purple very slowly, whilst in Suspension C the reduction occurred with the previous rapidity. At the end of 60 minutes, reduction had ceased in Suspension B, and all the parasites were dead; this suspension was then centrifuged at high speed and the supernatant fluid removed—'Extracted fluid B.' In Suspension C, reduction continued actively for another 2 hours; it then became slow and finally, after a further 30 minutes, ceased altogether, and practically all the trypanosomes were dead. Suspension C was then centrifuged at high speed and the supernatant fluid, 'Extracted fluid C' removed.

The sugar content of each of the three extracts and of the original sheep serum was then estimated, with the following results:—

- Original sheep serum, 51 mgm. per 100 c.c.
- 'Extracted fluid A,' less than 10 mgm. per 100 c.c.
- 'Extracted fluid B,' less than 10 mgm. per 100 c.c.
- 'Extracted fluid C,' less than 10 mgm. per 100 c.c.

The capacity to sustain trypanosomes of the original serum, of the three extracts, and of the various modifications of them shown in Table IV, was examined in the usual manner.

The results of the experiment recorded in Table IV show once again that the primary change produced in serum by the action of numerous trypanosomes is the loss of glucose. Serum treated in this manner (Extract A) quickly loses its capacity to support trypano-

somes, and that this is mainly due to lack of glucose is seen from the restorative action of the addition of 0.1 per cent. glucose. When, however, the serum has been subjected to still more prolonged action of the parasites (Extract B and Extract C), the addition of 0.1 per cent. glucose, although it restores in very large measure the nutrient properties of the serum, apparently fails to do so completely, with the result that trypanosomes added to such media die more quickly than in untreated sheep serum.

TABLE IV.

Tube		Number of living trypanosomes per 256 squares of the haemocytometer scale									
		Start	$\frac{1}{2}$ hour	1 hour	1 $\frac{1}{2}$ hours	3 hours	5 hours	7 hours	9 hours	12 hours	26 hours
1	Extract A		64	62	54	33	19	17	12	12	0
2	Extract A + 0.1% glucose	59	64	74	77	94	98	21
3	Extract A 1 part, sheep serum 1 part	72	...	81	75	...	96	22
4	Extract A 19 parts, sheep serum 1 part		60	...	61	35	15	11	19	11	0
5	Extract B		41	4	0
6	Extract B + 0.1% glucose		61	60	...	75	85	91	84	104	3
7	Extract B 1 part, sheep serum 1 part ...	62*	52	...	68	61	82	96	...	88	1
8	Extract B 19 parts, sheep serum 1 part		45	...	45	27	15	12	4	0	...
9	Extract C		47	5	0
10	Extract C + 0.1% glucose		69	74	...	59	71	64	62	80	4
11	Extract C 1 part, sheep serum 1 part ...		66	...	58	70	70	70	89	126	1
12	Extract C 19 parts, sheep serum 1 part		59	...	49	30	22	1	3	0	...
13	Saline 1 part, sheep serum 1 part ...		78	71	86	70	110	114	52
14	Sheep serum		58	78	86	93	89	102	72

* Average count of 6 tubes taken at random.

Considering, as a whole, the results obtained from the various combinations of 'Extracted' and normal serum, there appears to be substantial ground for believing that the prolonged sojourn of numerous trypanosomes in serum has some effect on its capacity to

support the parasites beyond that resulting from the mere removal of glucose, and that this additional action is probably the result of the removal of some other nutrient material rather than the production of toxic substances. We have no knowledge of the nature of this additional substance, which is of importance to the life of trypanosomes, but the comparative failure of various modifications of Ringer's solution to support trypanosomes (Tables V and VI), to which reference will shortly be made, suggests that it is of the nature of protein.

Summing up our observations on this subject, we have reached the conclusion that the primary cause of the rapid death of the trypanosomes in concentrated suspensions is the exhaustion of the glucose content of the serum. Probably, however, other factors, e.g., the exhaustion of other essential constituents of the serum and possibly the excretion of auto-toxins by the parasites themselves, play a secondary rôle in the phenomenon. It must, moreover, be remembered in this connection that pronounced agglomeration of the parasites into large masses is an invariable occurrence in concentrated suspensions; and as such masses rapidly subside to the bottom of the tubes, the accumulation of the vast majority of the parasites into a relatively small portion of the nutrient medium doubtless facilitates the operation of these inimical factors. We have frequently observed that when the clumping of the parasites into large agglomerations is prevented and the trypanosomes maintained in even suspension by frequent agitation of the medium, their length of life is considerably prolonged.

The result of this work has consequently shown that, provided the initial concentration of trypanosomes in the rabbit, or sheep, serum does not exceed about 1,000 per c.mm., i.e., between 60 and 70 per 256 squares of the haemocytometer scale, the parasites survive in practically undiminished numbers for a period of 24 hours at 37° C. After this period, the number gradually falls, but if precautions are taken to exclude bacteria, living parasites capable of infecting mice may frequently persist in considerable numbers for three or four days. It was subsequently found that the serum—fresh, or deactivated at 56° C. for half an hour—of ox, horse, and pig, proved just as efficacious as that of rabbit or sheep.

In striking contrast normal human serum was found to possess

definite trypanocidal action. *T. rhodesiense* and *T. equiperdum* are rapidly killed by human serum even in high dilutions, at 37° C.; but *T. gambiense* is apparently uninfluenced by human serum under similar conditions. This action of human serum is so striking and seems to have so important a bearing on the question of the epidemiology of human trypanosomiasis that we have decided to reserve further discussion of the matter to a separate paper.

Having reached this position as regards serum as a medium for maintaining trypanosomes alive at 37° C., attention was turned to other possible media. Experiments of a similar nature with saline, various modifications of Ringer's* solution, nutrient broth, and broth containing 0.2 per cent. glucose, showed that, in the absence of serum, none of these are capable of supporting the parasites for more than a few hours at 37° C. (Tables V and VI). These tables also illustrate the truth of our previous contention that when trypanosomes, in suitable concentration, are suspended in serum, there is, during the first 6 hours at least, a steady multiplication of the parasites, and that after 24 hours, they are to be found in numbers at least equal to those at the commencement of the experiment. Apparently dilution of serum with Ringer-glucose solutions, to the moderate extent shown in the tables, in no way impairs its capacity to support the parasites.

Why, after the initial increase, there should be a continual decline in the number of parasites until, after periods varying from 48 hours to 96 hours or more, all are dead, and why this process is more rapid in certain tubes than in others containing the same medium we are unable to explain. One factor which certainly is highly inimical to the life of the trypanosomes is bacterial contamination, and this is difficult to eliminate entirely from experiments of this kind. However, these questions are outside the scope of the problem we set ourselves—the discovery of a method whereby trypanosomes could be kept alive in undiminished numbers over a period of at least 24 hours at 37° C. We are satisfied that we have succeeded in finding a solution to this problem, and

* The constitution of the Ringer's solution was :—

Sodium chloride, 0.9 gm.
 Potassium chloride, 0.025 gm.
 Calcium chloride, 0.02 gm.
 Sodium bicarbonate, 0.015 gm.
 Distilled water, 100.0 c.c.

TABLE V.
Trypanosoma rhodesiense

Tube	Medium	Number of living trypanosomes per 256 squares of the haemocytometer scale							
		Start	1½ hours	3 hours	5 hours	6 hours	23 hours	30 hours	48 hours
1	Physiological saline		1	0
2	Physiological saline + 0.2% glucose		19	10	3	0
3	Ringer's solution		0
4	Ringer's solution + 0.1% glucose		20	9	2	0
5	Ringer's solution + 0.2% glucose		24	22	8	2
6	Ringer's solution + 0.5 %glucose		27	21	15	2
7	Nutrient broth		28	21	5	1
8	Nutrient broth + 0.2% glucose		32	20	6	1
9	Rabbit serum deactivated		31	...	35	...	24	14	6
10	Rabbit serum deactivated + 0.1% glucose	45	...	31	21	3
11	Rabbit serum deactivated + 0.2% glucose	29*	44	...	37	14	6
12	Rabbit serum 2 parts + Ringer 0.2% glucose 1 part...		42	...	26	13	1
13	Rabbit serum 1 part + Ringer 0.2% glucose 2 parts		34	...	30	16	0
14	Rabbit serum 2 parts + Ringer 0.5% glucose 1 part...		41	29	18	2
15	Rabbit serum 1 part + Ringer 0.5% glucose 2 parts...		39	41	20	0
16	Sheep serum deactivated		35	40	29	25	9
17	Sheep serum deactivated + 0.1% glucose	34	32	22	9
18	Sheep serum deactivated + 0.2% glucose	42	27	17	8
19	Sheep serum 2 parts + Ringer 0.2% glucose 1 part	44	45	43	34
20	Sheep serum 1 part + Ringer 0.2% glucose 2 parts	47	59	43	26
21	Sheep serum 2 parts + Ringer 0.5+ glucose 1 part	36	30	12	11
22	Sheep serum 1 part + Ringer 0.5% glucose 2 parts	47	40	37	34

* This figure is an average of the counts on 6 tubes taken at random.

TABLE VI.
Trypanosoma equiperdum

Tube	Medium	Number of living trypanosomes per 256 squares of the haemocytometer scale								
		Start	1½ hours	3 hours	5 hours	6 hours	8 hours	20 hours	30 hours	48 hours
1	Physiological saline		0
2	Physiological saline + 0.2% glucose		33	16	5	1	0
3	Ringer's solution		0
4	Ringer's solution + 0.1% glucose		33	15	11	5	4	0
5	Ringer's solution + 0.2% glucose		42	22	21	13	5	0
6	Ringer's solution + 0.5% glucose		28	32	22	9	2	0
7	Nutrient broth		33	30	18	4	1	0
8	Nutrient broth + 0.2% glucose		24	26	19	9	0
9	Rabbit serum deactivated		35	...	58	46	19	5
10	Rabbit serum deactivated + 0.1% glucose		40	...	50	39	24	11
11	Rabbit serum deactivated + 0.2% glucose		35	...	64	32	22	11
12	Rabbit serum 2 parts + Ringer 0.2% glucose 1 part	37*	44	...	74	62	29	1
13	Rabbit serum 1 part + Ringer 0.2% glucose 2 parts		37	...	58	54	30	2
14	Rabbit serum 2 parts + Ringer 0.5% glucose 1 part	64	...	52	34	5
15	Rabbit serum 1 part + Ringer 0.5% glucose 2 parts	68	...	57	28	1
16	Sheep serum deactivated	52	...	43	31	10
17	Sheep serum deactivated + 1% glucose	56	...	33	28	6
18	Sheep serum deactivated + 0.2% glucose	53	...	44	26	11
19	Sheep serum 2 parts + Ringer 0.2% glucose 1 part	47	...	45	32	9
20	Sheep serum 1 part + Ringer 0.2% glucose 2 parts	58	...	41	31	7
21	Sheep serum 2 parts + Ringer 0.5% glucose 1 part	50	...	37	52	10
22	Sheep serum 1 part + Ringer 0.5% glucose 2 parts	46	...	38	13	1

* This figure is an average of counts on 6 tubes taken at random.

that the method will fulfil our requirements in that it will enable us to examine the trypanocidal action of drugs and other substances *in vitro*.

SUMMARY

1. As a preliminary step in an investigation of the mechanism of the action of drugs in experimental trypanosomiasis, it appeared desirable to study the action of the drugs in question *in vitro*.

2. For this purpose it was obviously necessary to discover some means whereby pathogenic trypanosomes could be preserved alive *in vitro*, in approximately undiminished numbers, at 37° C. over a period of at least 24 hours.

3. The efforts of previous investigators in this direction had not met with much success. It was, however, generally agreed that serum was the best medium, and that it was much easier to keep the parasites alive at laboratory temperature than at 37° C. It is not possible to obtain from this work information having any pretence to quantitative value, and so far as work at the body temperature is concerned, the only statement we have been able to discover of any real value is that of Rothermundt and Dale (1912), who merely recorded that in guinea-pig serum they were able to keep trypanosomes alive for at least 5 hours; the important question whether the number of parasites decreased substantially during this period, or whether the parasites were present in the same number at the end of the period as at the beginning is ignored.

4. Our own experimental work showed that it is possible to maintain a trypanosome suspension alive *in vitro* at 37° C., without any appreciable diminution in the number of individuals, during at least the first 24 hours.

5. The method of preparing such suspensions and of observing changes in the number of parasites occurring in them, from time to time, is described.

6. It is shown that serum—fresh, or deactivated at 56° C. for half an hour—from the rabbit, ox, sheep, horse or pig, are about equally efficacious as supporting media, and that physiological saline, Ringer's solution—with or without the addition of glucose—nutrient broth and broth containing 0.2 per cent. glucose are comparatively useless.

7. Normal human serum, even in high dilutions, was found rapidly to destroy *T. rhodesiense* and *T. equiperdum* at 37°C. *in vitro*: it had, however, no trypanocidal action on *T. gambiense*.

8. It was further shown that the concentration of trypanosomes in the medium is a matter of vital importance. The parasites live longest provided their concentration does not exceed about 1,000 per c.mm.; if they are present in concentrations grossly exceeding this number, they die rapidly. The explanation of this fact is, doubtless, bound up with the great metabolic activity of the trypanosomes which, when the parasites are present in considerable concentration, rapidly deprives the medium of its nutrient properties and particularly of its glucose.

9. The presence of glucose is essential for the life of trypanosomes *in vitro*. Information is supplied concerning the relatively enormous quantity of glucose consumed by these parasites. It was found that 0.25 c.c. of heavily-infected blood mouse, containing approximately 400 million parasites, sufficed, when suspended in 5 c.c. of sheep serum, to which glucose had been added, to cause within 1 hour the disappearance of between 2 mgm. and 2.5 mgm. of sugar, and within 5 hours of between 12 mgm. and 12.5 mgm.

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STUMPY AND POSTERIOR-NUCLEAR FORMS IN A STRAIN (FEROX) OF *TRYPANOSOMA BRUCEI*

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There has long been considerable doubt about the relationship between the *Trypanosoma brucei* originally described by Plimmer and Bradford in 1899 and maintained in European laboratories since, and the polymorphic *T. brucei* or *ugandae* now met with in the field. Bruce had discovered a trypanosome in domestic stock suffering from nagana in 1895, and the figure of this parasite which he gave in 1897 showed it to be polymorphic. In 1899 trypanosomes, considered to be the same as that figured in 1897, were investigated in England by Plimmer and Bradford and named *T. brucei*. According to their description the trypanosome was monomorphic. Stephens and Fantham also described their strain, derived from that of Plimmer and Bradford, as monomorphic. Presumably the various laboratory strains of *T. brucei* have come from this original infection.

Differences in their immunity reactions described by Kroó and by Browning and Gulbrandsen are no doubt to be ascribed to the fact that the strains have been passed through different animal hosts at various times, and the infections have not been restricted to mice. At the early period of investigation on trypanosomes little attention was paid to such points as the influence of the host on the parasites, which may be very enduring. This first appeared from the work by Ehrlich, Roehl and Gulbrandsen on serum-fast strains.

It may therefore be of interest from the morphological point of view to record that in a 'ferox' strain of *T. brucei** received from Professor Mesnil, Institut Pasteur, Paris, in a guinea-pig sixteen months ago and since kept in mice at the Pathology Department, Glasgow University, the writer observed on several occasions

* This strain was obtained through the Medical Research Council.

stumpy forms. Among these there were six individuals showing various degrees of posterior nuclear displacement, one being a typical 'posterior-nuclear' form.

This typical posterior-nuclear individual was seen in the blood of a mouse in which the parasites were gradually disappearing as a result of the administration, 42½ hours previously, of the anil preparation No. 71 (Browning, Cohen, Ellingworth and Gulbransen). It had no free flagellum and the nucleus was displaced almost completely to the posterior end. The cytoplasm did not show the granular appearance sometimes seen after drug treatment. The other five were seen in the blood of a mouse which had been treated 45 hours before with the styryl preparation No. 90. Four of these were dividing forms, two nuclei being present, while the cytoplasm was still unsegmented. In these one nucleus was placed centrally while the other was almost or completely within the posterior quarter, that most displaced being about half its own diameter from the posterior end. The remaining specimen showed no signs of division; its nucleus was partly within the posterior quarter and was separated by a little more than its own length from the posterior end of the trypanosome. Though the axoneme was quite distinct there was little or nothing of the flagellum free. The cytoplasm showed coarse granules scattered throughout it.* In some of the other stumpy forms there were marked granular changes and neither axoneme nor undulating membrane could be seen, but in at least four specimens (one before and three after treatment) the axoneme was quite distinct though there was no free flagellum.

Altogether over 10,000 trypanosomes were examined.

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* The animals from which the specimens were obtained were killed several days later when the parasites, as judged by microscopical examination, had disappeared from the blood, thus the further course of the infections could not be determined.

AËDES (AËDIMORPHUS) APICO- ANNULATUS EDWARDS AND YELLOW FEVER : A CORRECTION

BY

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In our recent work, 'Insects, Ticks, Mites and Venomous Animals of Medical and Veterinary Importance,' Professor Patton and the present writer refer, on page 198, to Bauer's (1928) transmission of Yellow Fever by *A. (Aëdimorphus) apicoannulatus* Edwards. It now appears certain, however, from an examination of specimens kindly presented to the Liverpool School of Tropical Medicine by Mr. C. B. Philip, of the West African Yellow Fever Commission, that the species referred to by Bauer was not *apicoannulatus*, but a somewhat similar species, which I found to be quite distinct from Edward's species, when working in Sierra Leone, where both species occur. This species, which I named *occidentalis* (1926), was uncommon in Freetown, in comparison with *apicoannulatus*, but material identified by other workers from Nigeria and the Gold Coast, has invariably been found to be, in reality, *occidentalis*. Further, Mr. Edwards informs me that he has not seen *apicoannulatus* from Nigeria or the Gold Coast.

As the synonymy given below will show, these two species have been confused in the literature since 1917 and the paper, in which I distinguished *occidentalis*, has evidently been overlooked by recent workers. In this paper I described the larva of *apicoannulatus*, and pointed out that Ingram and Macfie's description, based on their Gold Coast material, applies to the larva of *occidentalis*, as I found by rearing such larvae to the adult state in Freetown. It should be noted that, in the table showing the larval differences on page 102 of my paper, the names of the two species are reversed.

The chief difference between these two species, as is noted in our book (1929), is the possession by *occidentalis* of conspicuous paired

patches of white scales on the antero-lateral margins of the mesonotum. Another obvious difference is the total absence of a pale band on the proboscis, the possession of such a band being a conspicuous feature of *apicoannulatus*, as shown in our figure on p. 289. The ♂ hypopygium also shows very marked differences, as shown in my paper (1926).

While this paper was in the press I discovered that the name *occidentalis* is preoccupied in the genus *Aedes* by an Australian species of Skuse, so that a new name will have to be given to the West African species. It is proposed, therefore, to rename it *Aedes* (*Aëdimorphus*) *stokesi* in honour of Dr. Adrian Stokes, who died of Yellow Fever while working with Dr. Bauer and Dr. Hudson on the experimental transmission of this disease to laboratory animals.

The synonymy of these species is now as follows :—

***Aedes* (*Aëdimorphus*) *apicoannulatus* Edwards (1912).** *Bull. Ent. Res.*
Aëdimorphus alboannulatus Theobald (1905).

***Aedes* (*Aëdimorphus*) *stokesi* nom. nov.**

Aedes (*Aëdimorphus*) *occidentalis* Evans (1926). *Ann. Trop. Med. and Parasitol.*

Aedes (*Aëdimorphus*) *apicoannulatus* Edwards (1923).

Aedes (*Aëdimorphus*) *apicoannulatus* Edwards (1925).

Ochlerotatus apicoannulatus Ingram and Macfie (1917).

Aedes (*Aëdimorphus*) *apicoannulatus* Bauer (1928).

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PATHOGENICITY OF *TRYPANOSOMA LEWISI* AND BLOOD SUGAR IN INFECTIONS WITH *TRYPANOSOMA LEWISI* AND *BARTONELLA MURIS RATTI*

BY

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(*Received for publication 9 September, 1929*)

A short time ago it was published by Linton (1929), in these ANNALS, that generally, in accord with our own experiences, he had found that the blood sugar level of normal and splenectomized rats was not lowered by infections with normal and non-lethal strains of *T. lewisi*. We have, however, experimented also with a strain of *T. lewisi* which had become virulent to such an extent, that it often produced a fatal infection in splenectomized rats. In such cases we have always found a final hypoglycaemia. The opinion of Linton is that the death of these animals was not caused by the infection with *T. lewisi*, but by an infection with *Bartonella*, which was responsible for the depressed sugar level.

He states, further, that it is very difficult to avoid infection with *Bartonella* in experiments with *T. lewisi* in splenectomised rats. But this is not quite exact. As ascertained by Mayer, Borchardt and Kikuth, the organic arsenicals, i.e., Neo-Salvarsan, Atoxyl, Tryparsamide, etc., are of high therapeutic value against *Bartonella muris ratti*. These drugs are, however, entirely ineffective against *T. lewisi*. There is at present only one drug, i.e., Arsenophenylglycin, to which *T. lewisi* is susceptible and by which it can be destroyed when employed in doses as indicated below. The opinion of Taliaferro (mentioned by Stratman-Thomas and Loevenhart (1928), that Arsenophenylglycin is not active against *T. lewisi* can only be explained by supposing that the preparations he used were not

identical with the ones employed by us (kindly placed at our disposal by the I. G. Farbenindustrie-Elberfeld). Reichenow and Regendanz, and Kikuth and Regendanz, have for several years employed this drug for experiments with *T. lewisi* and found it extremely efficacious even with doses of 0.10 grams to 0.17 grams per kilo body-weight. It is, therefore, easily possible to work with splenectomised rats in experiments of infections with *T. lewisi* as the infection with *Bartonella* can be eliminated by, say, Neo-Salvarsan, which does not influence *T. lewisi*.

In the work of Regendanz and Tropp, we have not called special attention to the fact, that the splenectomised rats have been treated with Neo-Salvarsan beforehand, for at the time we were making a statement on the sugar metabolism in Trypanosomiasis. But in a paper published at the same time by Regendanz and Kikuth, we mentioned this fact. Besides, we have always examined preparations of rat blood stained with Giemsa's solution, so that infections with *Bartonella* could not be overlooked.

The question whether *T. lewisi* can produce fatal infections in the rat, has kept turning up for years. Several experimenters who have been studying this form of trypanosomiasis intensively, have observed an occasional occurrence of pathogenic action. We need not go into the existing literature on the subject, which is well known. The strain of *T. lewisi*, with which we worked, had become so virulent by quick successive inoculations and cultivations in N-N-N-Agar, that even a non-splenectomised rat died of infection with this strain, as described by Reichenow and Regendanz. In the blood of this rat were dividing forms for eight days prior to its death, while normally the period of division is very much shorter.

But it must also be observed, that even in non-splenectomised rats an infection with *T. lewisi* may activate an infection of *Bartonella*. Mayer observed in a normal rat infected with *T. lewisi* the spontaneous appearance of *Bartonella*, having already (1921) discovered *Bartonella muris ratti* in chemotherapeutical experiments in normal rats infected with pathogenic Trypanosoma (*T. rhodes*). We, too, observed the occurrence of a strong infection with *Bartonella* in a non-splenectomised rat as the result of an infection with *T. lewisi*. As the spleen forms protective substances against *Bartonella* infection in rats, as shown by Mayer, Borchardt and Kikuth, as well as against

infection with *T. lewisi*, as shown by Regendanz and Kikuth, the activation of *Bartonella* infection is thus brought about by the fact that the spleen, taken up with the production of antibodies against *T. lewisi*, fails to cope with the *Bartonella* infection.

We have now examined the blood sugar of rats infected with *Bartonella*. The rats were splenectomized and after the blood had been strongly infected with *Bartonella*, we made the determination of blood sugar. The blood was obtained by decapitation and the glucose determined after Hagedorn-Jensen.

TABLE I.

Rats number	DAYS							Blood sugar in mgs. per 100 c.cms.	
	0	1	2	3	4	5	6		
1	Splenectomy...	o	o	o	+	+	...	Sacrificed. Not fed for 20 hours.	108
2	" ...	o	o	o	+	+	+	Sacrificed. Not fed for 20 hours.	109
3	" ...	o	o	o	+	Sacrificed.	119
4	" ...	o	o	o	+	+	...	Died spontaneously.	71
5	" ...	o	o	o	+	+	...	Died spontaneously. Not fed for 22 hours.	20

+ — *Bartonella* in the blood.

As shown in the table, we only found a depression of the blood sugar level in animals which had died of infection with *Bartonella* (Rats 4 and 5). The examinations could be made immediately after the death of these rats. A depression of blood sugar did not exist in the three other rats infected with *Bartonella* (Rats 1, 2 and 3). Our results herein correspond with those of Linton.

It must still be considered that, as Regendanz observed in Trypanosomiasis of large animals such as monkeys, there is a disturbance of the blood sugar metabolism during the course of the infection. That does not generally happen with rats, perhaps because in them the infection with trypanosoma takes too rapid a course to allow the onset of disturbances of organs controlling the glucose metabolism. Therefore, it may be that similar conditions exist in infections of rats with *Bartonella*.

CONCLUSION

In rats dying of infections with *Bartonella muris rattii* there occurs a final hypoglycaemia.

Trypanosoma lewisi may sometimes have a pathogenic action on rats in which there is a final hypoglycaemia.

It is easy to eliminate the *Bartonella* infection in splenectomised rats infected with *T. lewisi* by arsenical preparations, without influencing these latter parasites, as *Bartonella* and *T. lewisi* react differently to all known organic arsenicals except Arsenophenyglycin.

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